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THE ISOLATION OF SECRETIN—ITS CHEMICAL AND PHYSIOLOGICAL PROPERTIES.

By JOHN MELLANBY.

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THE properties of secretin were described by Bayliss and Starling(1) in 1902. Since that time very little additional information has been published regarding the properties of this remarkable substance. In 1912 Dale and Laidlaw(2) published a preliminary note on the preparation of secretin picrate from the duodenal mucosa by precipitation with mercury salts and picric acid. In 1917 Dalmau(3) obtained a secretin preparation of high potency and low toxicity by precipitating secretin solutions with nine volumes of acetone. In 1926 Luckhardt, Barlow and Weaver(4) prepared a highly active solution by a modification of the original Bayliss and Starling method. About 100 c.c. of 0·4 p.c. HCl are introduced into the closed excised duodenum of a dog, and the loop with its contents is incubated for a variable time at either 37° C. or room temperature.

Bayliss and Starling stated that the physiological properties of secretin were (1) a powerful secretory action on the pancreas, and (2) a feeble secretory action on the bilary apparatus of the liver. Various observers have assigned other properties to secretin. Abelous and Soula(5) state that secretin increases nitrogenous metabolism, the secretion of urine, and respiratory exchange. Pitcairn(6) observed that secretin acts as a diuretic, but only when prepared from the same species as the injected animal. Downs and Eddy(7) state that secretin augments the red and white cells of blood by its action on bone marrow and lymphoid tissues. This hæmopoietic property of secretin was also described by King(8). Further, according to Downs and Eddy, secretin has a powerful action on striated muscle since purified secretin, perfused through frog's muscle, increases the work done and lengthens the fatigue time. In regard to carbohydrate metabolism Downs and Eddy state that secretin causes an increased output of sugar from some source in the organism. On the other hand, Santos(9) states that secretin exerts a

hypoglycæmic action in man, dog, and rabbit. Lambert and Hermann (10) observed a transient hyperglycæmia followed by hypoglycæmia.

The isolation of secretin.

The method adopted for the isolation of secretin depends upon three observations: (1) secretin may be extracted from the intestinal mucosa by absolute alcohol; (2) precipitated bile acids adsorb secretin from solution; and (3) dilute acid precipitates secretin from aqueous solution.

In a series of papers (11, 12, 13, 14) proofs have been given that secretin is present in a preformed condition in the intestinal mucosa, and that powerful secretin solutions may be prepared by extracting the duodenal mucosa with absolute alcohol. Further, it has been shown that in the living animal the secretion of pancreatic juice is determined by the entrance of bile into the duodenum. An analysis of these phenomena showed that bile salts, when absorbed from the intestine, carry secretin contained in the cells of the duodenal mucosa into the portal blood and so to the pancreas. This deduction indicated that secretin and bile salts come into intimate relation with one another owing to their chemical or physical properties. The original method of Bayliss and Starling for the preparation of secretin extracts indicated that secretin is soluble in dilute acid. In point of fact secretin in aqueous solution is precipitated by dilute acid. For convenience the description of the method is divided into a series of processes. The difficulties which may arise at each stage are considered in a following section.

(1) The mucosa is scraped off a number of duodena. In these experiments pigs duodena were used. After grinding with sand, 250 grams of mucosa are extracted with a litre of absolute alcohol for 30 minutes at room temperature. The mixture is filtered through a coarse filter paper (Chardin) giving a clear yellow filtrate.

(2) This filtrate is distilled *in vacuo* to about 40 p.c. of its volume, *i.e.* until the liquid becomes opalescent and starts to froth. The solution is made up to the original volume with distilled water. This causes partial aggregation of the colloidal soaps and fats contained in the extract.

(3) The removal of fats and soaps is effected by means of $CaCl_2$ (0·01 *N*) at a temperature of about 20° C. For this purpose 1 c.c. of $CaCl_2$ (*N*) is added to each 100 c.c. of the slightly warmed fluid from (2). Within a few minutes the whole of the fats and soaps aggregate and form a well-marked precipitate which may be quickly filtered off by a coarse filter paper.

(4) The clear filtrate from (3) is cooled in an ice chest. Two c.c. of

10 p.c. bile salt (commercial sodium tauroglycocholate) are added to each 100 c.c. of the filtrate. After complete mixing, acetic acid to the extent of 0·2 p.c. is added. A small precipitate is produced on adding bile salt, but this precipitate is increased by the addition of the acetic acid. The precipitated bile acids adsorb secretin and carry it out of solution. Flocculation and settling of the precipitate occur more rapidly at low temperatures. Therefore, after addition of the bile salt and acetic acid, the mixture is left for one or two hours in an ice chest.

(5) The precipitate from (4) is obtained by centrifugalisation, and is dissolved in a small volume of absolute alcohol. Some of the precipitate does not dissolve. This may be removed by spinning and rejected.

(6) The alcoholic solution of secretin and bile acid is added to five volumes of acetone. After a short time a flocculent precipitate of secretin separates out and may be obtained as a compact mass by spinning in a high speed centrifuge. The precipitate is washed with acetone (twice) and dried with ether.

(7) Further purification is obtained by dissolving the precipitate from (6) in a small volume of water in which this secretin is freely soluble. Acetic acid is added to the solution to the extent of 0·1 p.c. The secretin is precipitated and may be obtained by spinning, and drying whilst in the centrifuge tube with acetone and ether. The final product is very active and has all the characteristic properties which are described in the following pages.

Notes on the various stages in the preparation of secretin. (1) Secretin is rapidly destroyed by autolytic and digestive enzymes. Therefore the duodena should be obtained from recently killed animals. (2) The optimum temperature for the extraction of secretin from the mucosa by alcohol is between 15° C. and 30° C. At low temperatures (5° C.) only a small quantity of secretin is extracted; at high temperatures (70° C.) a great part of the secretin is destroyed. (3) Secretin in alcoholic solution is rapidly destroyed at temperatures above 60° C. Therefore distillation of the alcoholic extract should be carried out, *in vacuo*, at a low temperature. Distillation may be taken below a 40 p.c. volume without injury to the secretin. (4) The precipitation of the suspensoid colloids of fats and soaps by $CaCl_2$ occurs more completely and rapidly at 20° C. than at 10° C. Therefore this process is facilitated by warming the solution. At this stage there is always some loss of secretin since $CaCl_2$ precipitates the bile salts which are always present to some extent in the alcoholic extract of the duodenal mucosa. These precipitated bile salts carry some secretin out of solution. The loss however is small—usually about 10 p.c. of the

total secretin contained in the original alcoholic extract. (5) The adsorption of secretin by precipitated bile acids requires care. Commercial sodium tauroglycocholate contains varying quantities of sodium glycocholate and sodium taurocholate depending upon the source of the original bile. These two salts possess very different properties as regards precipitation by acid. A solution of sodium glycocholate becomes opalescent and slowly precipitates on the addition of small quantities of acetic acid; a similar solution of sodium taurocholate is not precipitated by large quantities of acetic acid. Further, according to Hammarsten (18) taurocholic acid keeps glycocholic acid in solution and so prevents precipitation on acidifying a mixture of both salts. The precipitation properties of these two bile acids are too obscure to be discussed in detail in this paper. As a matter of routine a commercial preparation of sodium tauroglycocholate was used which readily precipitated under the conditions described in the method. This stage must be controlled by animal experiments until the optimum conditions for the precipitation of the bile salt and the consequent adsorption of the secretin from solution have been determined. The method described is adequate for all commercial preparations of bile which, when dissolved in water, become opalescent on adding acetic acid to the extent of 0·1 p.c. The aggregation of the bile acid-secretin adsorption compound is assisted by carrying out this process at a low temperature. (6) Secretin is readily adsorbed from solution by a large variety of substances. Hardened filter paper is a particularly active adsorbant of secretin. Therefore after precipitating secretin from the crude solution by means of bile acid it is essential to centrifuge and not to filter all subsequent solutions.

Methods of animal experiment. It is advisable when carrying out this process for the isolation of secretin to control all stages by animal experiment. In experiments on cats the following procedures have been used:

A nonvolatile anæsthetic (usually veronal 0·45 grm. per kg. of body weight) is administered subcutaneously three hours before starting the experiment. After this time the animal, if kept warm, is usually well under the influence of the anæsthetic. In some cases however it is essential to give chloroform during the operative procedures. Cannulæ are tied into the right femoral vein and gall bladder, in the latter case the cannula being of large size to permit the free flow of bile. A large dose of secretin (0·1 mgm.) is injected into the femoral vein. This causes the pancreas to secrete copiously and the viscid pancreatic juice present in the ducts is excreted into the duodenum. After five minutes a ligature is

placed under the common bile duct and pancreatic duct near their entrance into the duodenum. The pancreatic duct is opened by a fine incision—a correct incision being evidenced by the free flow of pancreatic juice from the cut surface. This flow of juice greatly facilitates the insertion of the cannula into the pancreatic duct, and the rapid flow of juice into the cannula indicates that the procedure has been correctly carried out. The ligature by which the cannula is tied into the pancreatic duct includes the common bile duct to prevent any bile entering the duodenum during the course of the experiment. The necessity for preventing the entrance of bile into the duodenum when standardising secretin preparations is evident from previous experiments in which it has been shown that the absorption of bile from the duodenum leads to a copious flow of pancreatic juice. The cannula inserted into the gall bladder allows the free exit of bile from the liver during the course of the experiment and is essential since bile is continuously secreted by the liver. Finally, it must be emphasised that cats vary considerably in their capacity to secrete pancreatic juice. Some cats have a small pancreas and secrete about 1 c.c. of pancreatic juice in 30 minutes; others possess a well-developed pancreas and may secrete 5 c.c. of pancreatic juice in that time. Therefore comparative experiments should be carried out on the same animal.

A protocol of an experiment is given to illustrate the method: 200 grm. of duodenal mucosa (pig) were ground up with sand and extracted with 800 c.c. of absolute alcohol for 30 mins. at room temperature (16° C.), 800 c.c. of filtrate were distilled *in vacuo* to 310 c.c. at a temperature not exceeding 50° C. This was diluted to 800 c.c. with distilled water and warmed to 20° C. Eight c.c. of $CaCl_2$ (*N*) were added, the fluid was well shaken and allowed to stand for five minutes. The precipitated fats and soaps were removed by filtration through a coarse paper. The clear filtrate was cooled to 5° C. and 16 c.c. of 10 p.c. sodium tauroglycocholate and 8 c.c. of 20 p.c. HA were added. The mixture was well shaken and allowed to stand in an ice chest for two hours. At the end of that time the precipitate was centrifuged off and dissolved in 20 c.c. of absolute alcohol. The portion of the precipitate which did not dissolve in the alcohol was separated by centrifuging and rejected. The alcoholic solution was added to 100 c.c. of acetone. A fine precipitate was formed and this was allowed to settle for one hour. At the end of this time the precipitate was obtained as a compact mass by spinning, and after twice washing with acetone was dried with ether. The weight of the precipitate was 15 mg. This precipitate was now dissolved in 15 c.c. of water in which it was completely soluble. Acetic acid was added to this solution to the extent of 0·1 p.c.

A precipitate was produced which, after 15 min., was obtained as a compact mass by spinning the fluid. This precipitate, after drying with acetone and ether, weighed 6·0 mg. The pancreatic activities of the various fractions were as follows:

1 c.c. of the original alcoholic extract gave 1·2 c.c P.J.
1 c.c. of the filtrate after removal of the fats and soaps by $CaCl_2$ gave 1·0 c.c. P.J.
0·05 mg. of the first dried precipitate gave 1·7 c.c. P.J.
0·05 mg. of the second dried precipitate gave 5·5 c.c. P.J.
0·017 mg. of the second dried precipitate gave 2·5 c.c. P.J.

On the assumption that the first and last solutions may be compared, it follows that 1 c.c. of the original extract contained $\frac{1}{110}$ mg. of secretin and that a gram of the original mucosa yielded $\frac{1}{30}$ mg. of secretin.

The chemical properties of secretin.

Secretin is an amorphous powder of a pale brown colour.

Solubility. Secretin slowly dissolves in water. Solution occurs more readily on adding a little sodium bicarbonate. This solution is a pale yellow colour even in concentrations as low as 0·05 p.c. The solution in water gives a stable froth on shaking. Secretin is insoluble in acetone, ether or absolute alcohol, but alcohol containing 5 p.c. of water dissolves a recognisable quantity of it.

Colour reactions. Secretin contains carbon, hydrogen, nitrogen, oxygen, sulphur and phosphorus. The colour reactions of secretin tested on a 0·5 p.c. solution gave the following results. *Biuret*: the colour developed was faint but positive. *Xanthoproteic*: this reaction was definitely positive. *Millon's* reaction was well marked as was also *Pauly's* reaction. *Glyoxylic*: this reaction was very indefinite but probably positive. The *Ninhydrin* and the *Molisch* reactions were both negative. After the oxidation of secretin by HNO_3 and H_2SO_4 the solution gave a well-marked phosphate, but only a faint sulphur reaction. These qualitative tests indicate that secretin is a polypeptide containing tyrosine and probably histidine together with a large amount of organic phosphorus and a trace of sulphur. The negative ninhydrin reaction suggests the absence of a free amino group in the molecule.

The precipitation of secretin. Secretin is precipitated from solution in water by saturation with ammonium sulphate. On this account it may be classed as a secondary albumose. Secretin dissolved in water is readily precipitated by small quantities of acetic acid (0·1 p.c.). It is also precipitated by picric acid and tannic acid. These precipitations are probably due to the reaction of the fluids and not to the formation of secretin

picrate or tannate. It is not practicable to precipitate secretin dissolved in water by alcohol or acetone, but secretin dissolved in 80 p.c. alcohol is precipitated by five volumes of acetone.

Hydrolysis by acid and alkali. Secretin dissolved in water may be boiled without any loss of activity. It is rapidly hydrolysed by dilute acid or dilute alkali. Seventy-five p.c. is destroyed when a solution of it in 0·1 p.c. HCl is heated to 100° C. for five minutes; similar heating in 0·1 p.c. NaOH results in its complete destruction.

Hydrolysis by trypsin and pepsin. Secretin is rapidly destroyed by both trypsin and pepsin. An equal volume of cat's pancreatic juice activated by enterokinase (trypsin) destroys secretin within five minutes at 37° C. Pepsin in 0·2 p.c. HCl also destroys it within a few minutes.

Destruction by autolytic enzymes. Secretin rapidly disappears when washed duodenal mucosa is ground up with sand and is incubated at 38° C. Probably this destruction is due to the autolytic enzymes contained in the cells of the mucosa. In conformity with this action of proteolytic enzymes it is found that secretin injected into the lumen of the duodenum rapidly disappears but causes no secretion of pancreatic juice. Probably these effects are due to the non-absorption of secretin from the lumen of the intestine by the cells of the villi and its digestion by the intestinal enzymes. The facts emphasise the impossibility of administrating secretin by the mouth.

Destruction by bacteria. Secretin, dissolved in water and sterilised by heat, preserves its activity for a considerable time. If the solution is left exposed to the air its activity usually disappears in the course of two days. This destruction is probably due to the growth of bacteria in the solution. Secretin dissolved in 85 p.c. alcohol preserves its activity for many days.

Relation of secretin to acids and bases. Certain indications suggest that secretin forms salts with sodium, magnesium and calcium. In the isolation of secretin the first precipitation with acetone takes place much more readily if magnesium and not calcium has been used to precipitate the fats and soaps. Also, secretin obtained after the use of calcium is more readily precipitated by acetone if a few milligrams of potassium oxalate are added to the solution. On the other hand, secretin in the presence of strong acids (HCl 0·2 *N*) appears to act as a base. Hydrochloric acid of this strength dissolves considerable quantities of secretin which cannot be precipitated from solution by means of acetone. This apparent capacity of secretin to act as an acid or base corresponds with the assumption of its polypeptide nature. Probably the absence of free amino groups and

the large content of organic phosphorus determine the acidic character of the compound.

Adsorption of secretin. Secretin, dissolved in water, is readily removed from solution by adsorption. In the preparation of secretin many attempts were made to obtain a non-pigmented product by means of charcoal. Charcoal, added to a neutral solution of secretin in water, adsorbs the whole of the secretin; in alkaline solution the quantity of secretin adsorbed by charcoal depends on the degree of alkalinity, the more alkaline the solution the less the amount adsorbed. The adsorption of secretin in acid solution by charcoal cannot be tested because secretin is precipitated from solution by small concentrations of acid. In those solutions in which partial adsorption by charcoal was produced the change in activity was proportional to the change in pigmentation. This result indicates that the active principle is a pigmented substance. Alumina adsorbs considerable quantities of secretin from solution in water. On extraction of the alumina with alcohol the secretin is recovered in its original pigmented form. In alkaline solution alumina does not adsorb secretin; in acid solution the acid alone precipitates secretin and thereby removes it from solution in water. Secretin is not adsorbed by precipitated cholesterol when an alcoholic solution of cholesterol is added to a solution of secretin in water. Hence secretin cannot be purified in a way similar to that described for insulin. Similarly attempts were made to purify secretin by adsorption with benzoic acid. Sodium benzoate was added to a secretin solution and acid was added to the mixture to precipitate the benzoic acid. Secretin however is precipitated from solution by much smaller quantities of acid than are required to decompose sodium benzoate and effect precipitation of the benzoic acid. Finally, it must be emphasised that hardened filter paper adsorbs secretin to a marked degree. Filtration through hardened filter paper often diminishes the activity of a solution by 50 p.c. This fact indicates the necessity of separating precipitates from secretin solution by centrifugilisation and not by filtration.

Crystallisation of secretin. Attempts were made to crystallise secretin. These experiments were carried out on two general lines in order to produce very slow precipitation of secretin: (*a*) adding minimal quantities of acetic acid to a solution of secretin in water, and (*b*) adding small quantities of acetone to a solution of secretin in 90 p.c. alcohol. No success was obtained by either method, the resulting precipitates being amorphous and pigmented.

The physiological properties of secretin.

Experiments have been made to determine the physiological actions of secretin. The results are briefly summarised.

Administration.

(*a*) *Intravenous injection.* In the following experiments secretin was dissolved in water and injected into the right femoral vein. The resulting secretion of pancreatic juice usually lasts about 30 minutes, but secretion may continue for an hour.

(*b*) *Subcutaneous injection.* In the anæsthetised animal a subcutaneous injection of secretin may produce a small secretion of pancreatic juice, but in many instances no result is observed. If, however, secretin is given subcutaneously to a cat 15 minutes before the administration of a volatile anæsthetic, then the pancreas yields a small secretion of juice when the duct is opened. It appears that secretin is absorbed slowly into the blood from the subcutaneous tissues. In the anæsthetised animal, which has been submitted to operative procedures, the rate of absorption may be too slow to stimulate the pancreas.

(*c*) *Alimentary administration.* The destruction of secretin by pepsin and trypsin is so rapid that it is useless to give secretin by the mouth. It was of interest to determine, however, whether secretin, admixed with bile, is adsorbed from the small intestine. To determine this point it was necessary to inject the mixture of bile and secretin into a portion of the small intestine in which the mucous membrane contains no secretin, since the absorption of bile only through the duodenum leads to a secretion of pancreatic juice. The ileum of the cat contains no secretin. Therefore a solution consisting of 2 c.c. ox bile, 7 c.c. Ringer and 1 c.c. $NaHCO_3$, 1·5 p.c. containing 1 mg. of secretin was injected into the ileum of a fasting cat in which cannulæ had been tied into the pancreatic duct and common bile duct. Within ten minutes the secretion of bile by the liver increased five-fold (due to the absorption of bile salts from the ileum). This increased secretion of bile continued for two hours. During that period there was no secretion of pancreatic juice and therefore no absorption of secretin, although the bile-secretin mixture contained as much secretin as about fifty maximal intravenously injected doses. The result indicates that the association of bile salt with secretin is very labile, since the bile salt was rapidly absorbed into the portal blood whereas the secretin remained in the ileum.

Pancreas.

(*a*) *External secretion.* The action of secretin on the external secretion of the pancreas has been discussed in a previous paper (16). Experimental results were given which indicated that the metabolism of the enzymes of the pancreas is controlled by the vagus nerves. Under the stimulus of secretin the cells of the pancreas secrete a dilute solution of sodium bicarbonate (approximately 0·15 p.c. $NaHCO_3$) by which the enzymes of the gland are carried into the intestine. This bicarbonate solution also provides the optimum medium for the enzymes to exert their digestive activities.

(*b*) *Internal secretion.* It has been stated by a number of observers that secretin acts on the cells producing the internal secretion of the pancreas; the evidence adduced being that secretin produces hypoglycæmia when injected into the intact unanæsthetised animal. These statements have been investigated in a number of experiments on rabbits. In the case of secretin preparations of a high degree of activity, large quantities (1 mg.), injected subcutaneously, have no influence on the glucose content of the boood. In some early experiments relatively impure secretin preparations, injected subcutaneously, produced a small fall in the blood sugar. Further purification resulted in the disappearance of this hypoglycæmic reaction. Probably a small quantity of insulin derived from the intestinal mucosa was associated with the secretin in the impure products. It may be definitely stated that the action of secretin is limited to the external secretion of the pancreas.

Liver.

The action of secretin as a cholagogue has been discussed in a previous paper (17). A series of experiments proved that this action of secretin is secondary to its action on the pancreas. As a result of the secretin stimulus products of pancreatic activity pass into the portal blood and stimulate the liver cells to secrete bile.

Red blood corpuscles.

A detailed investigation into the secretion of bile shows that many cholagogues have active hæmolytic qualities. In the early stages of this work various preparations of secretin were found to act as hæmolysins when added to suspensions of red blood corpuscles. With further purification however it was found that the hæmolytic property was lost whilst the cholagogue activity of the preparations remained.

Smooth muscle.

The results of many early experiments in which the secretion of pancreatic juice followed the absorption of bile from the duodenum indicated that secretin facilitated digestion by increasing intestinal peristalsis and by expelling bile from the contracted gall bladder (18). Therefore the action of secretin on the smooth muscle of the small intestine and uterus was investigated.

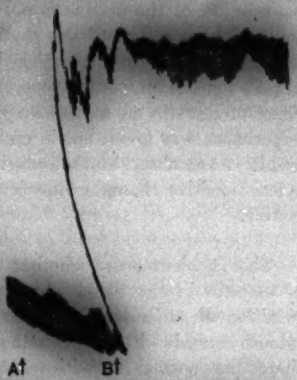

Fig. 1. 1 in. of jejunum of rabbit suspended in Tyrode solution (25 c.c.). *A–B*, normal rhythmic contractions. At the point *B*, 0·1 mg. secretin was added to the solution giving a concentration of secretin of 1 : 250,000.

Intestinal muscle.

One inch of the jejunum of a young rabbit was suspended in oxygenated Tyrode solution and the contractions were recorded by a lever. This portion of the rabbit's intestine shows marked rhythmic contractions as recorded by the tracing (Fig. 1 *A–B*). The contractions occur about every eight seconds, the rate of contraction being greater the less the amplitude of the contractions. At the point (*B*) 0·1 mg. of secretin was added to the Tyrode solution (25 c.c.), the concentration of secretin being 1 : 250,000. A large increase in the general tonus of the muscle was produced, this being followed by a return of the rhythmic contractions at the higher tonic level (Fig. 1). After the solution was removed and replaced by Tyrode solution the intestine returned within a few minutes to its original condition. Fig 2 shows the effect of adding 0·03 mg. of secretin to the Tyrode solution, the concentration of secretin in this case being 1 : 750,000. The resulting increase in tone was small, indicating that a concentration of secretin less than this quantity does not affect intestinal muscle.

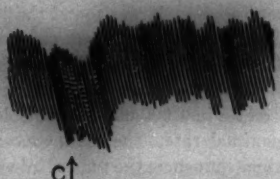

Fig. 2. Same as Fig. 1. At the point *C*, 0·03 mg. of secretin was added to the Tyrode solution giving a concentration of secretin of 1 : 750,000.

Uterine muscle.

The uterus of a guinea-pig was suspended in Tyrode solution. At the point (*A*), Fig. 3, 0·1 mg. of secretin was added to the solution. A large contraction of the uterine muscle resulted, followed by a return of the rhythmic contractions of the uterus which had been absent for the previous five minutes. The effect of secretin on uterine muscle in this experiment was great, but in many experiments it was observed that uterine muscle is less sensitive than the muscle of the intestine.

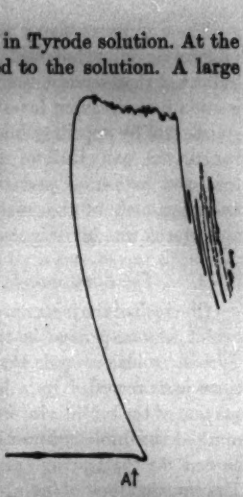

Fig. 3. Guinea-pig's uterus suspended in Tyrode solution (25 c.c.). At the point *A*, 0·1 mg. of secretin was added to the solution, giving a concentration of secretin of 1 : 250,000.

The problem arose whether quantities of secretin in the blood which produce a secretion of pancreatic juice also stimulate smooth muscle—in fact, whether secretin shares its normal activities between the pancreas and the plain muscle of the body. The effects produced by the intravenous injection of secretin into a cat showed that the pancreas is much more sensitive to the action of secretin than the muscle of the intestine. The pancreatic activity of the secretin preparation used in the plain muscle experiments recorded was determined. It was found that the intravenous injection of 0·03 mg. of this secretin into a cat resulted in a maximum pancreatic secretion, 3·6 c.c. of pancreatic juice being secreted in 30 minutes. The volume of the cat's blood was 150 c.c., so that a concentration of one part of secretin in 4,500,000 parts of blood caused the pancreas to secrete at a maximal rate. The least concentration of secretin in Tyrode solution which influences the tone of intestinal muscle is approximately 1 : 500,000. Therefore the pancreas is about ten times as sensitive to the action of secretin as the plain muscle of the intestine. A concentration of secretin in the blood which produces a maximal stimulation of the pancreas should have no action on the plain muscle of the body.

This experiment was confirmed by an experiment on the urinary bladder of an anæsthetised cat. A cannula, connected with a reservoir of warmed salt solution, was tied into the bladder. The rhythmic contrac-

tions of the smooth muscle of the bladder wall produced corresponding variations in the volume of the salt solution reservoir and these were recorded by a tambour. The injection of secretin (0·03 mg.) into the blood caused a copious secretion of pancreatic juice (3·2 c.c. in 30 min.) but did not contract or relax the bladder wall.

The question therefore arises whether secretin influences intestinal movements. Probably it produces increased tone locally when it is absorbed into the blood in the normal manner, that is, when bile salts are absorbed from the intestine and carry secretin from the intestinal mucosa into the blood. In this way secretin may be absorbed in a sufficiently concentrated form to act on the smooth muscle in its immediate vicinity and so produce increased intestinal movement.

No definite evidence has been brought forward to show that the secretin isolated by this method and used in these experiments is chemically pure. These two physiological actions therefore may indicate that the product contains two principles, the first (secretin) which excites the pancreas to activity, and the second which stimulates plain muscle. In the previous pages it has been shown that proteolytic enzymes rapidly destroy the pancreatic activity of secretin solution. This property was therefore used to investigate the above hypothesis. An equal volume of cat's activated pancreatic juice was added to a strong solution of secretin and the mixture incubated at 38° C. for ten minutes. After this time the solution had no action on the pancreas or the intestinal muscle. Sometimes a secretin solution loses its pancreatic activity after 24 hours owing to the growth of bacteria in it. This change is accompanied by a corresponding loss of activity on intestinal muscle. In fact, changes in pancreatic activity always proceed *pari passu* with changes in smooth muscle activity, indicating that the two properties depend on one principle only. Incidentally it may be observed that the action of secretin on the intestinal muscle of the rabbit in dilutions of 1 : 250,000 is much more intense than that of a corresponding quantity of pure histamine, the substance most likely to be associated with secretin.

Vascular system.

An intravenous injection of secretin contained in an alcoholic extract of the duodenal mucous membrane causes a profound fall of arterial blood-pressure. There is no relation between the fall of blood-pressure and the pancreatic activity of the solution; and it has been established by the work of numerous observers that the depressor principle has no relation to secretin.

In view of the action of purified secretin on plain muscle (intestine and uterus) it was of interest to determine whether a similar action could be observed on the plain muscle of the vascular system. For this purpose a Symes cannula was placed in the aorta of a frog and the vascular system perfused with Ringer solution from a constant pressure bottle. The drops of perfused fluid were registered on a slowly revolving drum. After the flow was established 0·1 mg. of secretin dissolved in Ringer was added to the perfusing fluid in the open limb of the cannula. For a brief period, about one minute, the rate of the flow was increased. The number of drops then progressively decreased until only one drop per minute of fluid passed through the perfused vascular system. The following is an abstract of such an experiment:

Normal rate of flow: 30 drops per min.
Immediately after adding secretin (0·1 mg.) to the perfusing fluid: 45 drops per min.
Five min. later: 15 drops per min.
Fifteen min. later: 5 drops per min.

It is evident that secretin, in relatively large doses, produces a marked constriction of the perfused vascular system of a frog. Small quantities (0·01 mg.) of secretin added to the perfusing fluid produce no change in the rate of flow. The intense vaso-constriction produced by large doses of secretin is annulled by adding amyl nitrite to the perfusing fluid. Probably therefore secretin acts directly on the plain muscle of the arteries. The result of these experiments indicated that the intravenous injection into a cat of a large dose of secretin would produce a rise of arterial blood-pressure. To test this hypothesis the carotid blood-pressure of a cat anæsthetised with urethane was recorded in the usual way, cannulæ having been previously inserted into the pancreatic duct and the gall bladder. The intravenous injection of 1 mg. of secretin (approximately ten times the quantity of secretin required to produce a maximal secretion of pancreatic juice) produced only a small transient fall and rise of blood-pressure. When the flow of pancreatic juice started—about one minute after the injection—the arterial blood-pressure returned to its original value and remained at that level for the following 50 minutes, during which time pancreatic juice was secreted. The result indicated that secretin injected into the blood is quickly removed from the circulation by the pancreas and so reduced to a concentration below that at which it acts on the plain muscle of the arteries. Purified secretin therefore has no effect on the general arterial blood-pressure. This fact is in agreement with the observation that the secretion of pancreatic juice which follows the injection of dilute bile into the duodenum (previously shown to be due to

the absorption of secretin into the portal blood) is not accompanied by any change in the arterial blood-pressure. Direct inspection shows however that the secretion of pancreatic juice caused by the action of secretin is accompanied by vaso-dilatation of the pancreas, an effect which is probably secondary to the action of secretin on the cells of the pancreas.

Respiratory system.

The intravenous injection of a crude extract of secretin produces a more or less prolonged period of apnœa followed by compensatory dyspnœa. Large doses of crude alcoholic secretin, or repeated small doses, may kill an animal by paralysis of the respiratory centre. These toxic effects are due to the impurities contained in alcoholic extracts. Purified secretin has no action on the respiratory centre as evidenced by changes in the depth or frequency of the respiratory rhythm. The result is interesting, because the secretion of pancreatic juice involves the withdrawal of a corresponding volume of approximately 0·15 p.c. $NaHCO_3$ from the blood.

Kidney.

In view of the action of the kidney in maintaining the neutrality of the blood, experiments were made to determine whether secretin influenced the volume or reaction of the urine produced during the active secretion of pancreatic juice. The details of an experiment are given:

Pancreatic juice and bile were collected from the pancreatic duct and gall bladder, the common bile duct being ligatured. Urine was collected from a cannula with a bulbous end tied directly into the urinary bladder. The bulbous end of the cannula almost filled the bladder. In this way variations due to differences in the volume of the bladder were excluded. Successive quantities of secretin (0·05 mg.) were injected intravenously every hour for four hours. During the first 30 minutes after each injection of secretin pancreatic juice and bile were freely secreted; in the second half hour only small quantities of pancreatic juice and bile were obtained, the stimulating action of the secretin being exhausted in the first 30 minutes. During the whole time urine was secreted at varying rates.

	Time (min.)	Urine	P.J.	Bile
0·05 mg. secretin	30	2·4	4·0	0·8
	30	1·6	0·1	0·0
0·05 mg. secretin	30	1·1	4·2	0·9
	30	0·6 *A*	0·0	0·0
0·05 mg. secretin	30	1·1	4·1	1·1
	30	1·0	0·0	0·2
0·05 mg. secretin	30	1·1	4·5	1·1
	30	0·7 *B*	0·1	0·1
Total quantities in four hours		9·6	17·0	4·2

An analysis of these results shows that secretin does not stimulate the kidney to secrete urine. In three out of the four hourly periods there was less urine secreted in the second half hour. This effect was probably caused by the diminution in the body fluid owing to the excretion of approximately 5 c.c. of pancreatic juice and bile during the first 30 minutes. During four hours 17 c.c. of pancreatic juice and 4·2 c.c. of bile were excreted. These secretions contained about 0·15 p.c. $NaHCO_3$ and represented a bicarbonate content of a corresponding volume of blood (21 c.c.). It was observed that all the specimens of urine had approximately the same reaction except (*A*) and (*B*). In these two periods smaller quantities of urine were secreted of a slightly greater degree of acidity than those produced during the other six half hour periods. Therefore the acidity of the urine appeared to be determined by its rate of secretion and not by the quantity of pancreatic juice secreted. There was certainly no evidence that, in the anæsthetised experimental animal, the kidney attempts immediately to preserve the neutrality of the blood by secreting urine of a compensatory reaction to that of the alimentary secretion.

Salivary, gastric and intestinal secretions.

Secretin has no action on the salivary glands, the gastric mucosa, or the glands of the small intestine. Crude alcoholic extracts of secretin may produce a secretion of gastric juice, but this effect was never observed with purified preparations of secretin. Probably some of the crude alcoholic extracts contained histamine which possesses a definite stimulating action on gastric secretion.

Voluntary muscle.

In view of the results of Downs and Eddy(7) the action of secretin on voluntary muscle was determined. There was no evidence that purified secretin exerts any action on the contractile properties of frog's voluntary muscle either by altering the form of the contraction curve or by delaying the onset of fatigue.

Summary.

(1) The method described for the isolation of secretin is based on, (*a*) the extraction of secretin from the duodenal membrane by absolute alcohol, (*b*) the adsorption of secretin by precipitated bile acids, and (*c*) the precipitation of secretin from aqueous solution by dilute acid.

(2) Secretin is a polypeptide containing phosphorus. It is slightly

soluble in water, freely soluble in dilute alkali, but insoluble in alcohol, acetone and ether.

(3) Secretin, in aqueous solution, is rapidly destroyed by (*a*) dilute acid and alkali at 100° C.; (*b*) pepsin, trypsin and autolytic enzymes, at 38° C.

(4) Secretin is adsorbed from aqueous solution by bile acids, charcoal, alumina, and hardened filter paper.

(5) Secretin acts most effectually when intravenously injected; it produces a slow secretion of pancreatic juice when administered subcutaneously to the unanæsthetised animal; it is not absorbed from the alimentary canal even in the presence of bile.

(6) Secretin acts on the external secretions of the pancreas and the liver. It has no action on the glucose content of the blood.

(7) Secretin increases the tone of smooth muscle (intestine, uterus, blood vessels) in a concentration of 1 : 500,000 approximately; but secretin in concentrations in the blood which produce a maximal secretion of pancreatic juice (1 : 5,000,000 approx.) does not influence the general plain muscle of the body.

(8) Secretin has no action in the arterial blood-pressure, although it produces local vaso-dilatation in the pancreas.

(9) Secretin has no action on the respiratory system or the voluntary muscle of the body.

(10) Secretin has no action on the secretion of urine by the kidney, except that the flow of urine decreases slightly immediately after the secretion of a large quantity of pancreatic juice and bile produced by the intravenous injection of secretin.

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THE CONCENTRATION AND DIFFUSION OF INORGANIC PHOSPHATE IN LIVING MUSCLE.

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Till recently our knowledge of the inorganic phosphate content of muscle has been very uncertain, for lack of an adequate method of analysis, by which to distinguish the organic from the inorganic fraction. Thus Costantino(1) gave the ratio of inorganic to total phosphorus as 81·5 p.c.: from this figure, and from the value of 0·1862 p.c. obtained by Katz(2) for the total phosphorus in fresh frog's muscle, the concentration of inorganic phosphorus could be calculated as 0·151 p.c. Embden(3) pointed out that the ratio of 81·5 p.c. given by Costantino was too high, owing to the presence of "lactacidogen" which breaks down and is erroneously estimated as inorganic phosphate by the method employed. Adler(4) found in the muscles of the frog's upper legs a concentration of phosphorus of 0·227 p.c., of which only 0·108 p.c. was present as inorganic phosphate, a ratio of about 50 p.c., much lower than Costantino's estimate. Embden and Hentschel(5) gave figures from frog's resting muscle varying from 0·073 to 0·101 p.c. of inorganic phosphorus, and Eggleton and Eggleton(6) found values, employing Embden's method, of 0·092 and 0·095 p.c.

All these figures are very high compared with those for blood, at any rate for human blood, where the concentration of inorganic phosphorus is of the order of 3·5 to 5 mg. per 100 c.c.: the difference could only be explained by supposing that the muscle fibres were impermeable to inorganic phosphate. Such an impermeability, however, was not in keeping with the fact established by Havard and Reay(7) that the concentration of phosphate in blood might change as the result of muscular exercise, and with the increased excretion of phosphorus in urine found by Embden and Grafe(8) under like conditions. Havard and Reay pointed out that the small rise in the phosphorus concentration of blood which took place during and immediately after exercise was followed by a rapid fall well below normal: they interpreted this fall as due to the

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absorption of phosphate from the blood by the muscles which had previously performed work. Their results seemed, indeed, to show that the muscle fibres are really permeable to phosphorus both in the resting condition and during activity and fatigue. Embden and Adler(9) had come to the same conclusion, from experiments made on the gastrocnemius of the frog kept in Ringer's solution: to explain the greater amount of phosphate appearing in the solution in a given time from a fatigued muscle, as compared with a resting one, they assumed that during activity and fatigue a considerable increase occurs in the permeability of the muscle fibre. This hypothesis seemed, at the time, the only possible one, since the normal concentration of inorganic phosphate in the resting muscle was supposed to be so high, and no considerable increase in its concentration as the result of fatigue had, as yet, been detected. As a matter of fact Parnas and Wagner(10) as well as Laqueur(11) had not been able to find any increase in the inorganic phosphate content of frogs' muscles after stimulation to complete fatigue. Only comparatively recently Embden and Schenkel(12) succeeded in showing an increase in fatigue, which, however, was in no case greater than 25 to 30 p.c. of the resting value.

A completely new outlook on the matter has recently been achieved as the result of the work of Eggleton and Eggleton(6, 13, 14), and of Fiske and Subbarow(15), who have demonstrated that in the resting condition the major portion of the supposed inorganic phosphate of muscle is in fact bound in an organic form, which is very unstable in acid solution, and breaks down during the estimation by the methods of Neumann, Embden or Briggs. It has been shown that this organic compound, to which the name "phosphagen" has been applied, breaks down largely in rapidly induced fatigue: in rigor it disappears completely. The figures for inorganic phosphate are more than doubled in fatigue, and multiplied by four in rigor.

The discovery of phosphagen and of its breakdown during activity made unnecessary any longer the hypothesis of Embden and Adler, that a considerable change in the permeability to phosphate is induced in the muscle fibre by activity and fatigue. A sufficient explanation of their results, and of those of Havard and Reay, would be the increase now known to occur in the inorganic phosphate content of muscle as the result of stimulation. One object of the present investigation was to determine whether, in fact, any such change of permeability does occur; it will be seen in the sequel that no evidence can be found of the existence of the alleged change.

Another object was as follows. All the methods employed for the determination of inorganic phosphate have involved the destruction of the tissue, a process which, in itself, may cause breakdown to occur before the final estimation is reached. In the present paper an attempt has been made to test the conclusions arrived at with such methods by one which involves no destructive manipulation of the tissue, and deals only with the phosphate which passes into, or out of, a solution with which the living muscle is brought in contact. This examination has led to the result that the inorganic phosphate content of the intact muscle —or at any rate the concentration with which it is in equilibrium—is still lower than biochemical methods hitherto have given. The process investigated has been the diffusion of phosphate from, or into, a muscle kept in Ringer's solution containing phosphate at various concentrations. The method demands that the membranes of the muscle cell shall be permeable to inorganic phosphate, a fact established by Embden and Adler and confirmed in full in the present experiments. It requires also that the surface of the muscle fibres should be impermeable to phosphagen, supposing that substance to be free inside, which it is probably not: the inability of phosphagen to pass out has been proved by P. Eggleton in unpublished experiments which he has kindly allowed me to quote.

Experimental details.

English or Dutch frogs, *Rana temp.* or *Rana esc.*, were employed, the animals being kept for some days previously at 1° to 2° C. in a cold store. Throughout the work the same preparation was used, namely the upper legs carefully separated from the body and all adherent tissue at their upper end and at the knee joint. In every experiment the upper legs of three frogs, weighing 9 to 12 grm., were placed in 20 c.c. of Ringer's solution containing various quantities of a mixed solution of Na_2HPO_4 and NaH_2PO_4 adjusted to a pH = 7·2. A standard solution was prepared containing 1 p.c. phosphorus, and added in appropriate quantities to the Ringer's solution which was made with glass distilled water and had the following composition:

NaCl	0·65 p.c.
KCl	0·014 p.c.
$CaCl_2$	0·0125 p.c.
$NaHCO_3$	0·02 p.c.

Diffusion took place at 1·5° C. in a 120 c.c. cylinder, carefully stoppered at the top in order to prevent evaporation of the liquid and escape of the gas (oxygen or nitrogen).

When the resting muscle was being investigated the legs employed were put into the diffusion liquid as soon as possible after dissection, and oxygen was passed through the solution every half hour in order to maintain the oxygen partial pressure. When muscles in fatigue and rigor were being investigated, it was necessary to prevent oxidation and recovery after the beginning of the diffusion; for that reason they were kept, previous to stimulation, for an hour at 1·5° C. in Ringer's solution containing $M/500$ KCN; to avoid loss of phosphate during this time phosphate was added to the amount of 8 mg. of phosphorus p.c., a concentration which, as will be seen later, allows no diffusion of phosphate to take place from, or into, a muscle under resting conditions. Then the muscles to be fatigued were excited in air at 18° C. by short tetanic stimuli every one second, with an induction coil adjusted to a distance to give maximal, but not supermaximal, stimuli. The muscles to be put into rigor were kept in an atmosphere of nitrogen at 20° C. for 30 to 34 hours, until full rigor had developed. Then in either case, fatigue or rigor, the muscles were introduced into the diffusion liquid, which contained $M/500$ KCN.

At the beginning and at the end of the diffusion period samples of the Ringer's solution were taken and the concentration in them of phosphorus was determined by the Briggs method as modified by Eggleton and Eggleton. Finally the surface of the upper leg was estimated by multiplying the length by the average circumference, and the amount of phosphorus which had diffused in, or out, through one square centimetre of muscle surface was calculated. In all that follows—unless otherwise stated—phosphate will be measured in terms of milligrams of phosphorus and phosphate concentrations in milligrams per 100 c.c.

Results.

The amount of phosphate diffusing clearly depends upon the time allowed. For short periods of diffusion the muscle may be regarded practically as a "semi-infinite solid," that is to say as a solid unlimited inwards, in which case a simple mathematical equation applies,

$$\text{Amount diffusing} = 2c\sqrt{\frac{kt}{\pi}},$$

where the amount is reckoned in milligrams diffusing, in or out, per square centimetre of muscle surface, c is the initial difference of concentration between the muscle and the Ringer's solution expressed in milligrams per c.c., k is the diffusion constant, and t is the period of diffusion. We see that, provided the time be not too long, in which case

the assumption of the semi-infinite solid no longer holds, the amount diffusing should be proportional to the square root of the time; this theoretical relation has been approximately verified on a number of occasions, as shown in Table I.

TABLE I.

The relation between the amount of phosphate diffusing from, or into, a muscle and the time of diffusion.

(t_1 in each experiment is the shorter time, t_2 the longer time.)

No. of exp.	Concentration of phosphate in Ringer: Before, mg. p.c.	After, mg. p.c.	Time of diffusion, minutes	$\frac{\sqrt{t_1}}{\sqrt{t_2}}$	$\frac{P \text{ diffused in } t_1}{P \text{ diffused in } t_2}$	Remarks
1	—	0·158	5	$\frac{\sqrt{5}}{\sqrt{36}} = 0·37$	$\frac{0·158}{0·400} = 0·39$	Resting muscles
	—	0·400	36			
2	—	4·00	25	$\frac{\sqrt{25}}{\sqrt{125}} = 0·45$	$\frac{4·00}{8·70} = 0·46$	Rigor muscles
	—	8·70	125			
3	—	1·4	360	$\frac{\sqrt{360}}{\sqrt{1260}} = 0·535$	$\frac{1·4}{2·88} = 0·485$	Resting muscles
	—	2·88	1260			
4	50·00	46·00	260	$\frac{\sqrt{260}}{\sqrt{1380}} = 0·435$	$\frac{4}{7·5} = 0·53$	Resting muscles
	50·00	42·50	1380			

It will be seen that the amount diffusing in time t_1 bears to the amount diffusing in time t_2 the ratio of the square root of t_1 to t_2. Thus the above formula is obeyed within the limits of time considered in the present experiments.

The results of a series of experiments in which the concentration of phosphate in Ringer's solution, which is in diffusion equilibrium with the phosphate inside the muscle, has been measured, are given in Table II. Every experiment lasted for six hours, and in Fig. 1 is given graphically the amount of phosphate which has diffused in this time through 1 sq. cm. of muscle surface into, or from, a Ringer's solution containing phosphate in the concentration named. It appears that the diffusion process is a simple reversible one, the amount of phosphate which leaves the muscle through unit area in unit time decreasing with increasing concentration of phosphate in the Ringer's solution; at a certain concentration no diffusion occurs, while at higher concentrations phosphate passes from the solution into the muscle.

On the hypothesis of free diffusion the relation between amount diffusing and concentration in the Ringer's solution should be a linear

TABLE II.

Showing the amount of phosphate which diffuses in six hours at 2° C. through 1 sq. cm. of muscle, at various concentrations of phosphate in the Ringer's solution.

Mg. of P p.c. in Ringer: Before diffusion	Mg. of P p.c. in Ringer: After diffusion	Surface of muscle (sq. cm.)	Total amount of P diffused (mg.)	Amount diffused through 1 sq. cm. (mg.)	Remarks
—	1·3	50	+0·26	0·00520	Rest
3·86	4·52	56	+0·132	0·00230	,,
6·00	6·52	50	+0·104	0·00208	,,
8·00	8·00	56	—	—	,,
12·00	11·14	60	−0·17	0·00280	,,
16·00	15·1	48	−0·18	0·00375	,,
30·00	27·25	52	−0·550	0·01800	,,
50·00	45·23	50	−0·95	0·01900	,,
—	2·26	50	+0·452	0·00900	Fatigue
7·00	7·95	45	+0·189	0·00420	,,
27·30	26·40	50	−0·180	0·00360	,,
50·00	46·5	48	−0·700	0·01440	,,
—	15·75	43	+3·15	0·0730	Rigor
32·00	43·20	44	+2·23	0·052	,,
64·00	72·50	43	+1·7	0·039	,,
64·00	71·00	40	+1·4	0·035	,,

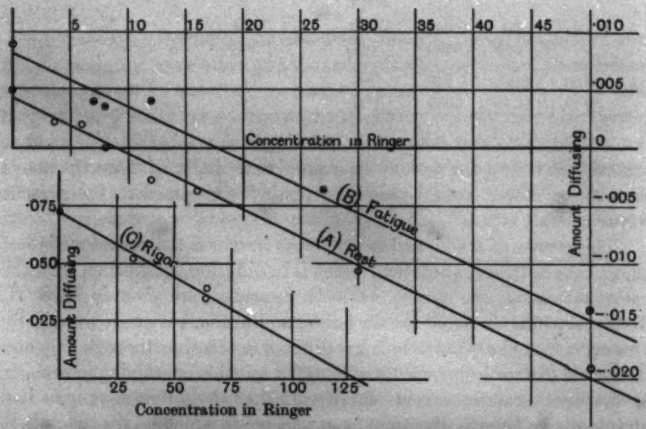

Fig. 1. The relation between the amount of inorganic phosphate diffusing out from, or in to, a muscle and the concentration of inorganic phosphate in the Ringer's solution with which it is in contact. (*A*) resting muscle; (*B*) fatigued muscle; (*C*) muscle in rigor, with the coordinates plotted on one-fifth of the scale. The concentrations of inorganic phosphate are given in mg. of phosphorus per 100 c.c. The amount of phosphate diffusing is given in mg. per sq. cm. of muscle surface, per six hours, at 2° C. For further details see text. Note that the three lines are approximately parallel, showing that the diffusion constant is nearly the same in all cases, slightly greater in the last.

one. This is verified, at any rate approximately, by the graphical representation of the results in Fig. 1.

The concentration of phosphate in the Ringer's solution, which is in equilibrium with the inside of the muscle, is dependent on the condition of the latter; in a resting muscle it is of the order of 8 to 10 mg. p.c., in a fatigued muscle it is 18 to 20 mg. p.c., and in a muscle in rigor it is 120 to 130 mg. p.c.

It might be suggested that these values refer to an equilibrium of phosphate between the Ringer's solution on the one hand and the liquid, blood or lymph, on the other hand, which is present in the interspaces between the muscle fibres, and that from such experiments we get no idea of what is happening inside the muscle fibres themselves. The same objection might be made to the experiments of Embden and Adler(9), which could be interpreted on the hypothesis that during activity, and only during activity, the cell membrane becomes permeable to phosphate, which passes into the interspaces and from these, later on, to the Ringer's solution. To answer this objection it is necessary to prove that in the diffusion experiments phosphate has been derived from the inside of the fibres, or, alternatively, that phosphate has penetrated them from the Ringer's solution. If it be agreed that a certain degree of permeability is possessed by the sarcolemma, it follows that the process of equalising the concentration around a given fibre with the lymph in its neighbourhood must be extremely rapid owing to the small dimensions of the fibre and the extreme thinness of the sarcolemma. Let us assume that the interspaces between the fibres occupy one-fifth of the volume of the whole muscle—a liberal estimate: we may then show that the suggestion that we are dealing only with the interspaces of the muscle must be false. A series of experiments was performed in which diffusion was allowed to proceed for a much longer period than in the experiments described above. Either the Ringer's solution contained no phosphate, or it contained 50 mg. p.c. It was maintained carefully oxygenated throughout the period of diffusion in order to keep the muscle in good resting condition, and at the end of the experiment the excitability of the muscle was tested and found to be well preserved. Assuming that the concentration of phosphate in the interspaces of the resting muscle is 8 mg. p.c., we see from Table III that the amount of phosphate which has left, or penetrated, the muscle in 22 to 24 hours is larger than could be derived from the whole of the interspaces in the case of diffusion outwards, or could be contained in the interspaces at the full concentration of the solution itself in the case of diffusion inwards.

TABLE III.

Showing the volume of muscle required to give out, or to take up, all the phosphate which diffused into, or from, the Ringer's solution, assuming that the concentration of phosphate in the muscle fibres and in the interspaces is 8 mg. p.c. The volume of the interspaces in c.c. is taken as 20 p.c. of the weight in grm.

No. of exp.	Initial phosphate concentration in Ringer (20 c.c.)	Final P concentration in Ringer	Time of diffusion (hours)	Total amount of phosphate diffusing (mg.)	Weight of muscles (grm.)	Volume of interspaces (c.c.)	Volume of muscle required to give out, or to take up, the phosphate diffused from, or to, the muscle (c.c.)
1	—	2·88	21	+0·575	12	2·4	7·2
2	50·00	40·00	24	−2·00	7·5	1·5	6·2[1]
3	50·00	41·60	24	−1·68	10	2	5·00[1]

[1] This value is obtained by dividing the amount of P diffused by the difference between the P final concentration in Ringer and the initial concentration in the muscle assumed to be 8 mg. p.c.

In Table III is shown the volume of muscle which would be required to give out, or to take up, all the phosphate found by observation to have diffused. No reasonable estimate of the interspaces could account for the volume required, so we may conclude that the phosphate in these experiments is derived from, or penetrates into, the muscle fibre through the sarcolemma. Once inside the sarcolemma it is difficult to imagine that the phosphate cannot permeate freely the major bulk of the whole tissue, though there may still be restricted regions into which it is not able to pass.

The results of Table II and Fig. 1 allow a calculation to be made of the absolute value of the diffusion constant k. In the equation,

$$\text{Amount diffusing} = 2c\sqrt{\frac{kt}{\pi}},$$

if the time t be expressed in minutes, the amount in mg., and the initial concentration in mg. per c.c., the constant k is obtained in the usual units of amount diffusing per sq. cm., per minute, per unit gradient of concentration. The ratio, $\frac{\text{amount diffusing}}{2c}$, can be read off very accurately from the slopes of the lines in Fig. 1: it is the same for the resting and the fatigued muscles[1]: expressed in the above units it is 0·0241. In all the experiments t was 360 minutes. Substituting we find,

$$k = 5{\cdot}06 \times 10^{-6}.$$

[1] In the rigor muscles the ratio is 0·028, which seems to indicate an increase in the permeability: the difference, however, is too small to be decisive.

The diffusion constant of sodium phosphate unfortunately is not given in tables of physical constants. In Landolt-Börnstein, however, we find for sodium carbonate a value of $2{\cdot}7 \times 10^{-4}$, when expressed in the above units, and the value for sodium phosphate cannot be far from this. To obtain, however, an approximate experimental figure for the diffusion constant of phosphate, experiments have been made with a solid agar-agar jelly (1·5 p.c.) in Ringer's solution. The jelly was formed in the bottom half of a beaker and contained phosphate at *p*H 7·2 at various concentrations. Ringer's solution was placed above the jelly and diffusion took place from the jelly into the Ringer. The surface of the jelly was measured and the diffusion constant k determined from the formula above. The value so obtained is almost certainly too small—owing to the relative volumes of jelly and Ringer, and the rapidity of diffusion as compared with the case of muscles; the figure, 9×10^{-5}, should probably be at least three times larger. The experiments, however, were sufficient to verify the fact, on which the following argument rests, that *diffusion of phosphate through free solution is far more rapid than through muscle.* The blood and lymph in the interspaces of the muscle should provide about the same diffusion constant as the jelly. The much lower value obtained for the muscle as a whole suggests that diffusion through the surface of the fibres is relatively slow, and that in dealing with diffusion through the muscle in bulk the chief process involved is a diffusion round and about the fibres, and not mainly across them. So long as the sarcolemma has any permeability at all phosphate will rapidly come into diffusion equilibrium on its two sides: diffusion through such small distances as are here involved must be very rapid provided that the permeability of the sarcolemma, and of other membranes possibly involved, is not vanishingly small. If, however, the diffusion constant through the lymph were (say) 100 times as great as directly across the intact fibre with all its interfaces to hinder the progress of the ions and molecules involved, then further diffusion outwards would take place mainly by the devious path afforded by the lymph spaces between and around the fibres. Thus the diffusion constant observed through the muscle in bulk would be considerably less than that through a jelly, as has been found. In Fig. 1 the lines drawn through the observations (*a*) on the resting, (*b*) on the fatigued, and (*c*) on the rigor muscle are approximately parallel—the diffusion constant is nearly the same in all three cases. If we assume that the greater part of the diffusing phosphate actually passes straight through the muscle fibres on its course outwards (or inwards), then the equality of k in the three cases shows that the

"permeability" is practically the same for muscles in the three conditions. If, however, we imagine that diffusion takes place mainly through the interspaces, and that penetration of the fibre is relatively slow, then the results recorded here give us no information at all about the question of whether the permeability of the surface of the fibre is altered by stimulation or in rigor. Clearly if the diffusion constant of phosphate through the sarcolemma were 1/100, or 1/1000, or even 1/10,000 of that obtaining in free solution, it would nevertheless be possible, owing to the extremely small dimensions[1] of the system involved, viz. the single fibre, for the lymph around the fibre to remain approximately in diffusion equilibrium with the inside, and the diffusion constant through the muscle in bulk would remain the same in each case, since the ions and molecules would continue to pass mainly through the lymph in the interspaces. Only if the constant of diffusion through the fibre were of the same order of size as that of diffusion in free solution could we expect to find changes by methods involving diffusion through the muscle in bulk. The low diffusion constant observed shows that this is not so, and we may conclude that *no evidence exists for, or against, the view that a change of permeability occurs when a muscle is fatigued; the hypothesis that such a change occurs is unnecessary.*

It follows from the facts recorded above that, independently of any supposed changes in permeability during fatigue and rigor, it is possible for the lymph, and the Ringer's solution around the muscle, to come into equilibrium with the phosphate inside the muscle fibre, and that the values of the equilibrium concentration are given approximately by the points where the three lines of Fig. 1 cross the horizontal axis. For the resting muscle this equilibrium concentration of inorganic phosphate is 8 or 9 mg. p.c., for the fatigued muscle it is about 18 mg. p.c., for the rigor muscle about 130 mg. p.c. It is not by any means necessary that in the equilibrium condition there should exist the same concentration of phosphate inside and outside the muscle fibre. First of all, our equilibrium concerns only the freely diffusible inorganic phosphate, and the possibility may not be neglected that a part of the total inorganic phosphate may be in some form which is not mobile, for example, adsorbed or reversibly combined, and in equilibrium with the freely diffusible fraction. On the other hand, a membrane equilibrium of some kind might keep the concentration of inorganic phosphate inside and outside the sarcolemma at different levels. This might happen, for example,

[1] The time required for the equalisation of concentration by diffusion varies as the square of the distance involved.

were there a Donnan equilibrium at the fibre's surface, the phosphate being able to diffuse freely as an anion, but potassium and sodium unable to diffuse as kations. In this particular case, the analogy of the chloride concentration, which is about five times as great outside as inside, would lead us to expect that the phosphate would be *less* concentrated inside the sarcolemma than in the lymph. Without further information it is impossible to answer the question, what is the actual concentration of inorganic phosphate inside the muscle fibre? It is in equilibrium, across the membrane, with 8 or 9 mg. p.c. at rest, and with about 18 mg. p.c. in fatigue: but it does not follow that these are the actual concentrations in the fibres under such conditions. It will be seen that the present experiments confirm those of Eggleton and Eggleton in the sense that the resting content is low, being doubled or more than doubled in fatigue, but my values differ from theirs in absolute size, being considerably smaller. Since these experiments were concluded Mr Eggleton, in this laboratory, in unpublished experiments, employing an improved technique for obtaining an absolutely resting condition of the muscle, has succeeded in driving the inorganic phosphate concentration to values as low as 10 or 12 mg. p.c., which are not so much larger than those found for the resting muscle in my experiments. As regards fatigue, the difference still remains high: whereas the present method shows about 18 mg. p.c., the Eggletons do not find values lower than about 50 mg. p.c. It is possible that a different condition, determining the membrane equilibrium at the surface of the fibre, exists in fatigue which may account for this discrepancy. Perhaps between phosphagen and inorganic freely diffusible phosphate there is some intermediate form which is not diffusible, but is estimated as inorganic phosphate by the method employed by the Eggletons. For the muscle in rigor the results given here agree with those of the Eggletons.

Summary.

1. Inorganic phosphate is able to pass freely by diffusion into or out of a muscle immersed in Ringer's solution.

2. The relation has been found and plotted in Fig. 1 between the amount of phosphate diffusing out from, or into, a muscle when kept in contact with Ringer's solution containing phosphate, and the concentration of phosphate in the solution.

3. The relation is a linear one, and the amount diffusing is proportional to the square root of the time, which shows that the ordinary diffusion laws are obeyed.

4. The diffusion constant calculated from these results is the same (*a*) for a resting muscle, and (*b*) for a fatigued muscle, and nearly the same (*c*) for a muscle in rigor. It is very small when compared with that for free diffusion in water or jelly. In all probability diffusion takes place mainly through the lymph spaces of the muscle, diffusion through the fibre itself and across its various interfaces being slow. The small dimensions of the fibre enable equalisation of concentration between inside and outside to occur rapidly, but the phosphate having once passed out the course of its diffusion is probably mainly through the lymph around and between the fibres.

5. There is no evidence for any change in the permeability to phosphate of the muscle fibres. Such an hypothesis is unnecessary. The fact that phosphate diffuses more rapidly from a fatigued muscle, and far more rapidly from a muscle in rigor, is due simply to the greater concentration of phosphate in these two cases. The conclusions of Embden and Adler, regarding the supposed change of permeability in fatigue, were arrived at before the existence of phosphagen was realised.

6. The inorganic phosphate concentration in resting muscle is in equilibrium with between 8 and 9 mg. p.c. of phosphate in the lymph, and in the Ringer's solution with which the muscle is brought in contact. In the fatigued muscle the equilibrium occurs at 18 mg. p.c., and in the rigor muscle at 130 mg. p.c.

7. The concentration of inorganic phosphate inside the fibre is not necessarily the same as that in the external solution with which it is in equilibrium. A membrane equilibrium may occur, or adsorption, or reversible combination, may render part of the inorganic phosphate inside the fibres incapable of diffusion.

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THE EFFECT OF PREGNANCY AND MENSTRUATION ON THE SIZE OF THE SPLEEN.

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THE present research commenced in a somewhat accidental manner. Among the first dogs the spleens of which were exteriorised (1) was one which only at first responded well to exercise. Unlike others operated upon at the same time, the spleen after about a month became pale and small, presenting at rest the appearance which it had previously presented after exercise. It was killed by bleeding; the hæmorrhage caused little further contraction of the spleen—again unlike other animals which we had observed. The post-mortem which was performed by Sgt-Major Secker, R.A.M.C., revealed the fact that the animal was in an advanced stage of pregnancy.

Three further experiments have been performed, each of which was along the following lines. Two female dogs were selected of about the same size, one which when she "came on heat" was served with a dog. About half-way through the pregnancy, *i.e.* four to five weeks after the service, the spleens of both animals were exteriorised and the changes in each noted from time to time. As an account of these experiments appears in the *Archiveo di Scienze Biologiche* (in the complementary volume to Prof. Bottazzi(2)), and as now this may be regarded rather as preliminary, they will only be referred to rather shortly here:

(1) The spleen of each animal, the pregnant and the control, gradually gets smaller as the days pass, but the spleen of the control animal does not shrink nearly so rapidly as that of the pregnant bitch.

(2) This rapid shrinking of the spleen of the pregnant bitch goes on until about the time the pups are born. The third experiment, that in which the technique was most perfect, suggested that minimal shrinkage, and certainly the most obvious pallor of the organ were not coincident with the date of parturition, but took place two or three days earlier.

A record of one of these experiments is shown in Fig. 1, in which the area of the pregnant animal's spleen is shown as a percentage of the area of the spleen of the control animal on the same day. While they were

quite definite as far as they went, they left several points to be desired, both as regards technique and interpretation, of which the following three are the most important:

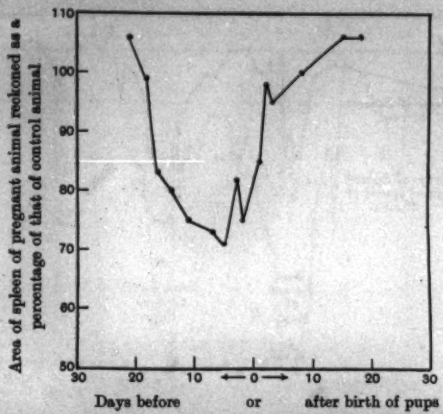

Fig. 1. "The area of the spleen" in pregnant animal expressed as a percentage of that in control animal. The spleens were exteriorised on successive days.

(1) The control spleen was not of uniform size through the experiment, the difference in size between the control and the normal was quantitative only and not qualitative.

(2) The animals all experienced a certain amount of anæmia, and broadly speaking the pregnant animals suffered most from anæmia; might it be that the shrinkage of the spleen was really due to the anæmia?

(3) Further as regards the interpretation, even granting that the contraction of the spleen was caused by a call for blood elsewhere, was that call due to the necessity of producing iron for the blood of the fœtus, or was it due to the enlargement of the capacity of the vascular bed caused by the congestion of blood in the genital organs?

A single experiment went far to settle all the points enumerated above. (This experiment has now been duplicated on another animal in all important respects excepting the hæmoglobin estimations which it seemed unnecessary to repeat.)

A bitch, the spleen of which was exteriorised on June 13th, 1927, was kept under observation, and by September 24th the spleen had ceased

to get smaller; from that date until January 9th, 1928, a period of about fifteen weeks, the spleen had scarcely varied in area and certainly it had not shrunk; the progressive alterations may be followed in Fig. 2.

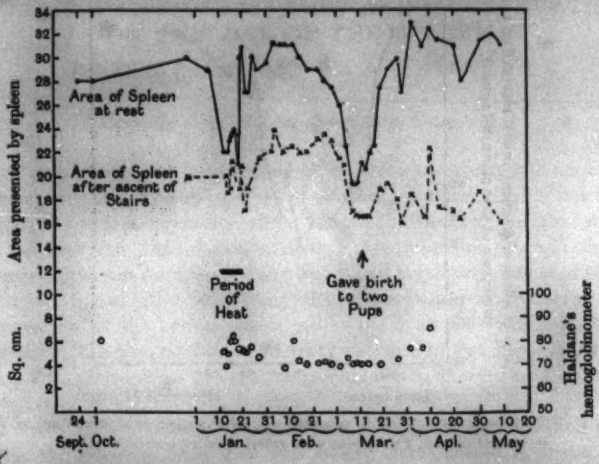

Fig. 2. Alterations in "spleen area" ⟷, at rest; ×—×, after standard exercise; and o o, hæmoglobinometric reading during menstruation and pregnancy.

The animal then came on heat with the result that the spleen contracted suddenly to about three-quarters of its area and remained so during the whole period of heat. Directly that period was over the spleen returned to its former area, or indeed attained a slightly greater one. About the middle of the period of pregnancy (February 12th) the spleen commenced again slowly to contract, reaching its smallest dimensions on March 7th-9th, the pups being born on March 12th. By April 2nd the spleen was larger than it had been at any measurement since August 19th of the previous year. The approximate alterations in volume are shown in Fig. 3, the assumption being that the alterations in volume are proportional to the square in the alteration in "surface" (1).

Applying the results of this experiment to the three points set forth above:

(1) The contraction due to pregnancy was not one which took place in a spleen which would in any case have been contracting, but at a lesser rate. It took place in a spleen which otherwise was of almost

constant size or, if anything, was growing larger. (On June 18th, 1928, it had an area of 37·5 square centimetres.)

(2) The hæmoglobin values were noted right through the experiment (see Fig. 2), and there are no such variations as could be held to account

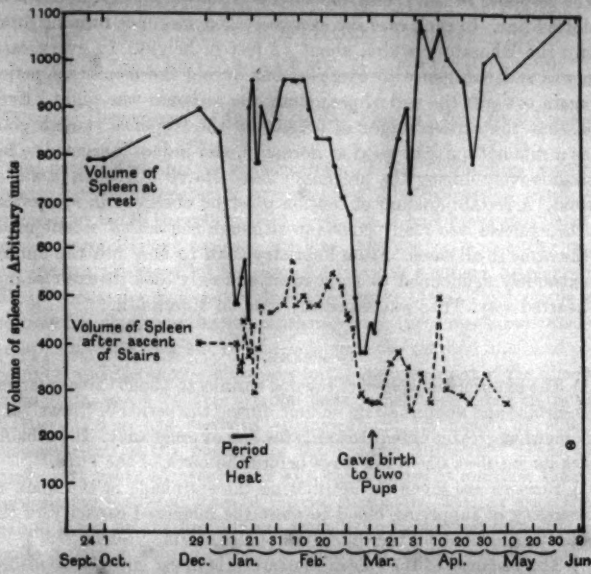

Fig. 3. Computed volume of spleen ←→ at rest and after standard exercise ×→•×→•× during menstruation and pregnancy. ⊗ after chasing cat along passage. The mass of the spleen in grams is not very different from the figure obtained by dividing the arbitrary units on the ordinate by fifteen.

for the alterations in volume of the spleen. Moreover it has been shown by Shen(3) in work which was being carried on at the same time that anæmia, as opposed to ischæmia, does not cause a shrinkage of the spleen.

(3) There is no question of considerable blood destruction during the period of "heat," therefore as between the rival hypotheses that the contraction of the spleen is directed towards (*a*) the making good in the circulation of blood destroyed for the purpose of supplying the fœtus with iron, and (*b*) the supply of blood to fill freshly opened vascular areas. The latter in the period of "heat" is the only one that need be considered.

Presumably therefore in pregnancy the enlargement of the vascular bed is the principal cause of the contraction of the spleen. The effort to make good to the circulation any actual loss which may take place by destruction of corpuscles is not ruled out as a concurrent factor.

The response of the spleen to exercise is shown in Figs. 2 and 3 by the dotted line. In each case the exercise taken was once running down and up the laboratory stairs, about 42 feet in height. In every case, there was some response to exercise, but during the menstrual period and again towards the end of pregnancy the response was slight. Even throughout the earlier stages of pregnancy the response though good was as a rule not quite so good as normally, and indeed it seemed to fall off progressively during the pregnancy until March 5th when it was at its worst. A certain amount of caution must be observed in suggesting that the exercise was really constant although the actual ascent made was the same in all cases. From February 24th to May 5th the animal was extremely disinclined to exert itself and only took its exercise in a half-hearted way. That was especially true on March 5th.

SUMMARY.

(1) The exteriorised spleen of the dog shrinks to about three-quarters of the surface and about half its volume during the period of "heat" and to a somewhat greater extent towards the end of pregnancy. It probably reaches its smallest size a few days before the birth of the pups.

(2) Reasons are given for attributing the shrinkage in the main to the necessity of supplying blood to meet the increased capacity of the vascular bed, caused by the dilatation of the uterine vessels.

(3) The response of the spleen to exercise is never entirely abolished during "heat" or pregnancy, though it is much reduced at the times when the spleen is shrunken.

(4) The colour of the spleen undergoes remarkable alterations, becoming pale during the periods when the spleen shrinks. If anything, the alteration in colour anticipated the alteration in size both when the spleen is paling and when the red colour is returning.

(The expenses of the above research have been borne largely by a grant from the Royal Society.)

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THE MEASUREMENT OF THE DIAMETER OF ERYTHROCYTES BY THE DIFFRACTION METHOD.

By ALEXANDER ALLEN and ERIC PONDER.

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This paper describes a modification of the diffraction method of measuring the diameter of erythrocytes. The diffraction method was introduced by Pijper(1) in 1919, although it had previously been used by Young. Pijper employs a white light source, and either projects the spectra produced by the film of cells upon a screen, or photographs the spectra and makes measurements from the plate. The formula which he uses for obtaining the cell diameter from these measurements is only an approximate one, but he has developed the method in a most elegant manner, and has extended its uses to the measurement of the dimensions of bacteria, etc.(2, 3). This original method has been much improved by Millar(4), who has designed an instrument by means of which the measurement of the position of the various diffraction bands can be made with great accuracy, and who has supplied in addition the correct formula for the calculation of the cell diameters. Millar finds that the values given by the formula are from 10 to 20 p.c. too high, and at the same time is unable to detect the change in diameter of the cells which occurs when they dry(5). He concludes that the method is not a reliable means of determining the absolute size of red cells, and that although small changes in size can be detected, the extent of these changes in absolute units must remain a matter of doubt. Millar's paper also contains a most valuable discussion of the limitations of the method and of some of the difficulties encountered.

Recently a simple modification of the diffraction method has been described by Emmons(6). The spectra are produced by a film of dried cells which is inserted into a small instrument (an "eriometer"), supplied with a white light source and with a simple device for determining the size of the various diffraction bands. Emmons claims that this method gives results correct to 0·1 μ, and that no change in diameter of the cells can be observed when they dry(7).

1. Method.

The principle of the method to be described is that the diffraction spectra are produced by passing monochromatic light through the film of red cells, the monochromatic light being derived from the various lines of the mercury arc.

The diffractometer used is one constructed exactly as described by Millar, but with two slight modifications in the telescope. (i) Instead of the opaque half diaphragm in the telescope eyepiece, this eyepiece is fitted with two cross threads at 90°, oriented in such a way that the one always transects the image of the pinhole while the other lies tangentially to the various diffraction bands of the spectrum. When the telescope is in the zero position the intersection of the two threads appears in the middle of the image of the pinhole. These cross threads can be adjusted more accurately to any desired position than can the opaque diaphragm, and in this method there is little glare from the central image. (ii) The clamping screw of the telescope is replaced by a rack and pinion by means of which the position of the telescope can be adjusted to 0·1° on the scale.

The instrument is enclosed on three sides so as to cut off all scattered light, and is adjusted exactly as described by Millar. It may be mentioned that the position of the spectra is not very sensitive to slight alterations in the distance between the pinhole and the collimating lens of the instrument.

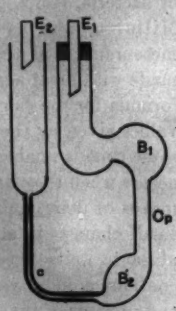

Fig. 1.

The source of illumination employed is a mercury vapour arc of special design, the appearance of which is shown in Fig. 1. The lamp is constructed from quartz tubing of about 1 cm. bore. This tubing is drawn out into a capillary *Cp* about 2·5 cm. long and 3 mm. in internal diameter, within which the arc is formed; at either end the capillary opens out into bulbs B_1 and B_2, one of which communicates with an exceedingly narrow capillary c. The purpose of this latter constriction is to damp the oscillations of the arc. Electrodes E_1 and E_2 are inserted into the open ends of the tube, one of the electrodes being held in position by a cork. The whole tube is filled with mercury. The electrodes are attached to a 110 volt D.C. circuit with a 60 watt lamp in series, and the arc formed by heating the capillary *Cp* with a Bunsen flame. It is desirable to have a small

fan in the neighbourhood of the arc to keep it cool, and under these circumstances it will burn steadily for hours. Should it go out, it usually re-forms spontaneously as it cools.

The intensely luminous filament which appears in the capillary *Cp* is now focussed on the slit of a spectrometer. In this way the spectrum of the mercury arc is obtained, and by means of a lens inserted between the prism of the spectrometer and the pinhole of the diffractometer, any one of the lines of the spectrum can be focussed on the pinhole of the latter instrument. Considerable care is necessary in the adjustment and alignment of the various parts of the diffractometer and spectrometer, but once these preliminary adjustments have been made it is only necessary to start the arc before use.

Before passing to the detailed consideration of the results obtained by the method, it may be as well to describe briefly the appearances seen in the telescope of the diffractometer under different conditions of illumination, when the apparatus is properly adjusted and when a thin dried film of human red cells is placed on the diffractometer stage.

(i) The principal lines of the Hg spectrum are the two yellow lines with wave lengths of 5770 and 5791 Å.U. respectively, the green line with a wave length of 5461 Å.U., the blue line at 4358 Å.U., and the violet line at 4078 Å.U. It is both difficult and unnecessary to separate the two yellow lines, which may be treated as a single line with a wave length of 5780 Å.U.; the violet line yields spectra to which the retina is comparatively insensitive, and thus we are left with three effective lines, the yellow, the green, and the blue.

When the film of dried red cells diffracts the approximately monochromatic light supplied by the two yellow lines, the pinhole appears brilliantly lit with yellow light and surrounded by a circular area of considerable intensity. There is, however, none of the glare associated with the light from the carbon arc, and it is easy to look directly at the image of the pinhole on which the cross threads intersect when the telescope is in the zero position. This yellow circle is surrounded by the first diffraction minimum, which appears as a circular dark band with fairly well-defined edges. This band is quite black at the points where the illumination is least; the illumination within it increases from its centre outwards, and undergoes a rather sharp increase at the edges. Surrounding this diffraction minimum is the first diffraction maximum, a yellow band of considerable luminosity. This is again surrounded by the second minimum, the position of whose edges is difficult to determine, and this again by the second diffraction maximum, a yellow band which

can only just be seen. It is quite easy to set the cross threads at the points in the maxima and minima where the illumination is greatest or least respectively, but the edges of the bands are a little deceptive, for the illumination changes rapidly at a point a little within the true edge of the band.

When green light is diffracted, the picture is much more brilliant. The first minimum is very intense, and the point within it at which the illumination is least can be readily determined. The first maximum is a brilliant green circle; the second minimum can be easily recognised, and so can the fainter maximum beyond.

The diffraction pattern with monochromatic blue light is equally clear, and at first sight even sharper than that with green light. The first and second maxima are completely black, while the two visible maxima are of an intense dark blue colour. Experience shows, however, that it is not so easy to set the cross threads in definite positions within the bands as it is in the case of green light, and that reliable measurements can be obtained only from the positions of the first minimum and of the first maximum.

The violet line of the Hg spectrum also gives a diffraction pattern which shows a faint first minimum and a fainter first maximum, but the sensitivity of the retina to this colour is so low that the necessary measurements are unobtainable. The same remark applies to the pattern seen with the red line of the mercury arc.

(ii) When the diffraction pattern is examined with the light of the mercury arc itself instead of with the monochromatic light derived from its various lines, a series of alternating yellow and blue bands appear. No distinct minima can be seen, for the yellow and blue bands overlap to some extent, nor is there any sign of the green diffraction maximum which should correspond to the green line of the arc spectrum. The limits of the yellow and of the blue bands can be measured with a considerable degree of accuracy, and such measurements bring out two very interesting points.

The first point is that there is only an approximate correspondence in position between the yellow band of the arc spectrum and the first yellow maximum with monochromatic light, and a scarcely closer correspondence between the blue band of the arc spectrum and the first blue maximum with monochromatic light. This will be seen by an inspection of Fig. 2, in which the position of the respective bands is shown; it will be observed that (*a*) the yellow and the blue bands of the arc spectrum do not correspond in position, except roughly, to the yellow and blue

first maxima with monochromatic light, and (*b*) that the green first maximum is missing from the diffraction pattern obtained with the light of the mercury arc as a whole. Measurements of the position of the limits or of the points of greatest luminosity within the bands of this latter diffraction pattern would accordingly give incorrect values for the diameter of the cells for no other reason than that the position of the bands is replaced from the true one owing to colour-mixing and overlapping.

The second point of importance is that there appears in the diffraction pattern obtained with the light of the mercury arc as a whole a very bright yellow band which has no apparent counterpart in the patterns obtained with monochromatic light. The position of this band, marked *B*, is indicated in Fig. 2, and it will be seen that it lies nearer

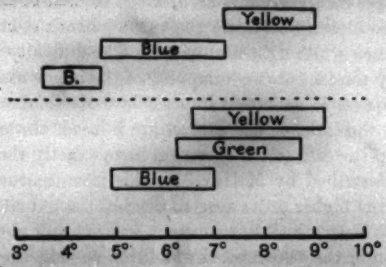

Fig. 2. Diffraction bands produced by human red cells. The positions of the 1st maxima for the monochromatic light of the blue, green, and yellow lines of the mercury arc are shown below the dotted line. Above the dotted line are shown the bands which appear when the same preparation of cells diffracts the light of the whole mercury arc. The band *B* is yellow in colour and corresponds to no maximum for monochromatic light. The green band which appears with monochromatic light is missing from the diffraction pattern.

to the axis of the optical system than does either the first blue band of the mercury arc diffraction pattern or the first blue maximum obtained with monochromatic light. In appearance it is absolutely indistinguishable from a diffraction band, but its position alone indicates that it cannot be one, for it lies nearer the optical axis than does the first diffraction band of a shorter wave length. Calculation of the position of the maxima and minima for the various wave lengths which make up the light of the arc shows that this yellow band really corresponds to the first minimum for the blue and violet lines of the arc, and that its appearance is due to the blue and violet components of the composite

light falling to a minimum intensity in this position. That this explanation is correct can be shown by inserting a potassium bichromate filter in the path of the light; this procedure causes the yellow band *B* to disappear altogether, for potassium bichromate cuts out the blue and violet lines of the mercury arc and therefore removes their first minimum. At the same time it allows the yellow lines to pass, and the fact that the band *B* disappears under these circumstances is proof that it is not a true diffraction band at all.

In the diffraction pattern obtained with the light of the mercury arc as a whole we have therefore the remarkable occurrence that a yellow band, superficially indistinguishable from a yellow maximum, represents a first blue-violet minimum. The conclusion to be drawn from the fact that such a misleading appearance occurs, even when light of a comparatively simple composition is used, must be that it may be difficult or impossible to identify correctly the various bands seen in the diffraction pattern when white light is employed. The difficulty will, of course, be increased by the fact that overlapping of the bands must occur under these circumstances to an extent impossible to determine.

(iii) When the light of the carbon arc is used, the coloured bands which make up the diffraction spectrum have exactly the same appearance as that described by Millar. As this investigator observes, the violet ends of the higher order spectra overlap the red ends of the lower ones, and it is very difficult to form a satisfactory judgment of the correct position of the telescope. In addition to this, the colour mixing and the comparative faintness of the spectra, due principally to the flooding of the field with white light, make it difficult to determine the exact wave length of the light which is being subjected to measurement. As Millar points out, an improvement can be effected by the use of filters. We have used for this purpose (*a*) an eosin filter which passes the yellow portion of the spectrum, (*b*) a double filter of neodynium chloride and potassium bichromate, which passes the green part of the spectrum only, and (*c*) the Zettnov filter used by Millar. The use of these filters purifies the diffraction spectra considerably, but even under these circumstances it is difficult to determine the position of the various bands. The difficulty is increased by the fact that the whole field becomes flooded with light of the same colour as that of the filter.

Emmons uses in his eriometer a source of white light, and his apparatus is therefore working under the greatest possible disadvantage[1].

[1] Emmons figures one diffraction pattern obtained with monochromatic light, but uses white light for his measurements.

The spectra obtained are certainly brilliant, but they are very impure, the impurity arising from the fact that the colours overlap as a result of the cells not being all of the same size. Under these circumstances it must be a very difficult matter to determine which particular wave length is being used in making the measurements. In addition to this, the diffraction pattern contains a yellow and an orange band lying within the first violet maximum, bands which are spurious in the sense that the band *B* is spurious. How many other such bands contribute to the impurity of the spectra is impossible to say. The eriometer would be greatly improved if an eosin filter were included in its construction, for this at least gives a series of comparatively pure yellow maxima and minima.

Just as the position of the maxima and minima supplies us with a measure of the mean size of the red cells of the population which produces the diffraction pattern, so the variation in the illumination intensity within the various maxima and minima supplies us with data regarding the scatter of the population about the mean. The study of this aspect of the problem, however, involves the use of special mathematical and experimental technique, and will not be considered in this paper.

2. The applicability of the theoretical formula.

Millar gives as the relation between the diameter of the cells and the position of the bands of the diffraction pattern

$$2a = z/\pi . \lambda . \operatorname{cosec} \theta .$$

Here a is the radius of the cell of average size, θ the diffraction angle, and z/π a constant for any one maximum or minimum. When applied to measurements made in conjunction with white light from the carbon arc, Millar finds that the expression gives results from 10 to 20 p.c. too high.

In order to show that this formula is more strictly applicable when monochromatic light is used, we give below a table of results obtained from the diffraction pattern produced by dried human cells. The film of cells was made in the usual way and allowed to dry in the air of the room; it was then placed on the diffractometer stage and the measurements made without it being moved. After completion of the measurements, the size of the cells was determined photographically in the usual way. The mean diameter was found to be $7{\cdot}6\mu$.

		θ	M
Yellow	1st minimum	5·3	7·7
	1st maximum	7·2	7·5
	2nd minimum	9·4	7·9
	2nd maximum	11·6	7·7
Green	1st minimum	5·2	7·4
	1st maximum	6·8	7·5
	2nd minimum	9·2	7·6
	2nd maximum	11·1	7·6
Blue	1st minimum	4·0	7·6
	1st maximum	5·2	7·8
	2nd minimum	7·4	7·6
	2nd maximum	8·9	7·6

Each one of these measurements was made for the point of greatest or least intensity within the maximum or minimum respectively, and all values for the mean diameter of the cells (M) are in μ. So far as these figures are concerned, it will be seen that single readings give results correct to within $\pm 0{\cdot}2\mu$, the average of a number of readings having, of course, a much higher degree of precision.

The following table gives a few values for rabbit cells, the mean diameter of which was ascertained by photographic measurement to be $6{\cdot}4\mu$.

		θ	M
Yellow	1st maximum	8·5	6·4
Green	1st minimum	6·1	6·3
	1st maximum	8·0	6·4
Blue	1st maximum	6·3	6·5

The next table gives a few values for the dried cells of the sheep. As soon as we come to cells so small as this, considerable difficulty is met with in the making of the films, for if the films are thick they dry to give exceedingly poor spectra, whereas if they are very thin the spectra have a very low intensity.

		θ	M
Yellow	1st minimum	8·6	4·7
Green	1st minimum	8·0	4·8
	1st maximum	10·9	4·7
Blue	1st minimum	6·6	4·6

The dried cells of this animal were found by direct measurement to have a mean diameter of $4{\cdot}7\mu$.

Finally, we give four values for the dried cells of *Hemitragus jemlaicus*, the diameter of which was found by direct measurement to be $3{\cdot}2\mu$. The cells of this animal are among the smallest of those of any of the deer family after acclimatisation at sea level.

		θ	M
1st minimum	Yellow	12·2	3·3
	Green	11·0	3·5
	Blue	9·0	3·4
1st maximum	Blue	12·3	3·5

It is, however, exceedingly difficult to obtain films which give a good diffraction pattern when the cells are so small as this, and even in the best films it is difficult to make out the limits of the various bands.

These results are sufficient to show that the diffraction method gives remarkably good results when monochromatic light is used, and that the formula given by Millar is applicable. When the diffraction pattern is obtained with the light of the whole mercury arc, however, the results are not nearly so good, for the coloured bands do not exactly correspond to the maxima given with monochromatic light, as Fig. 2 shows. When white light and the Zettnov filter is employed, we agree with Millar that the formula gives results which are too high by about 10 to 15 p.c., the discrepancy being no doubt due to the great difficulty in determining the predominant wave length and the position of the diffraction bands. White light and an eosin filter give somewhat better results, but even this combination falls far short of monochromatic light.

When white light is employed without a filter, as in Emmons' eriometer, it is a difficult matter to determine the wave length corresponding to the points of maximum intensity within the various diffraction bands. Emmons claims an accuracy of 0·1 μ, and gives a table in which the values for the diameter of the cells of four animals, as measured by diffraction, are compared with the diameter as measured by a projection method. The results agree very closely, the greatest error being only 1·8 p.c., but as we shall show below that the eriometer does not detect differences amounting to 10 p.c., the correspondence is to be regarded more as evidence of good fortune than as evidence of the accuracy of the method.

3. The effect of drying.

In discussing the applicability of the theoretical formula, we have confined ourselves to measurements of dried cells, for such measurements are not the subject of dispute. We have now to consider the statement of Emmons that the drying of erythrocytes is not accompanied by a diminution in diameter. This statement has been made by several observers, while a shrinkage has been claimed to occur by an almost equal number (8, 9, 10). So far as the recent observations go, the position may

be summarised by saying that Millar and one of us find by direct measurement that there is a 10 to 20 p.c. shrinkage on drying, and that Millar and Emmons, working independently, are unable to find evidence of shrinkage by the diffraction method. Millar concludes from this failure that the diffraction method suffers from imperfections, but Emmons, although he is aware of Millar's results and of his interpretation, concludes that only a negligible shrinkage occurs and that the direct measurements are unreliable.

We agree with Millar and with Emmons that it is not possible to detect a diminution in diameter on drying from the study of the diffraction pattern produced in conjunction with white light. When monochromatic light is used, on the other hand, the results are very different, for the drying of the cells is accompanied by a marked change in the position of the diffraction maxima and minima. This change can be followed as the cells dry, in the case of the cells of man and of the rabbit at least, and may be demonstrated in the following simple manner. A film of cells is made on a slide, and the film while still wet is placed on the stage of the diffractometer. A reading is made at once, the most convenient reading being that for the middle of the first maximum or of the first minimum. It is often possible to measure the position of the edges of the 1st maximum and minimum in addition. The film is then allowed to dry without being in any way disturbed, and readings again made; in this way the diffraction pattern for the wet cells is formed by the very same cells as contribute to the formation of the pattern for the dry cells. Results of this procedure are shown in the following table, the figures being for the middle of the first green minimum.

	θ wet	θ dry	M wet	M dry
Man 1	4·6	5·2	8·3	7·4
„ 2	4·5	5·0	8·5	7·6
„ 3	4·5	5·1	8·5	7·5
„ 4	4·5	5·0	8·5	7·6
„ 5	4·4	5·0	8·7	7·8
„ 6	4·3	4·9	8·9	7·8
„ 7	4·3	4·8	8·9	8·0
Rabbit 1	5·3	6·0	7·3	6·4
„ 2	5·5	6·1	7·0	6·3

These figures are sufficiently numerous to show that shrinkage takes place on drying, and it is, indeed, a simple matter to observe the first diffraction minimum moving outwards as the drying occurs. Since neither the diffractometer in conjunction with white light[1] nor the

[1] Millar, it should be noted, does not claim that his method can determine differences in absolute units.

eriometer of Emmons are able to detect this change, it is to be concluded that neither of these methods is sufficiently sensitive to demonstrate with certainty differences which amount to 10 or 15 p.c.

In connection with these results two points require to be emphasised. (i) The extent to which the cells shrink on drying varies somewhat, even under what appear to be the same conditions. This variation may be related to the fact that many films give a very poor diffraction pattern on drying, presumably owing either to irregular shrinkage of the cells or to numbers of the cells overlapping. This difficulty is particularly liable to be encountered if the films are made thick. (ii) Even when monochromatic light is employed, the diffraction method does not, in our experience, give satisfactory results for cells in plasma. Usually the diffraction bands are not quite sharp, and this gives rise to difficulties in making the readings. When the cells are suspended in undiluted plasma, rouleaux formation is very troublesome. When they are suspended in plasma diluted with NaCl, it is difficult to avoid crenation occurring. The best suspension medium is probably a mixture of plasma and citrate, as used by Millar, but even in this fluid some cupping of the cells is often apparent, and one cannot be certain that changes of shape and size have not occurred. Diffractometer measurements on such preparations generally show a slightly smaller diameter than that found by photography; in the case of human cells, for example, photography will show a mean diameter of 8·7 μ, while the diffraction method will show a diameter of about 8·3 μ. The latter method is accordingly not so reliable in the case of fresh preparations as it is in the case of dried films, the errors arising, however, from the difficulty in obtaining satisfactory preparations.

We have not been able to demonstrate shrinkage on drying in the case of the cells of the sheep, goat, or *Hemitragus* when diffraction methods are used. The difficulty here consists in making really satisfactory films which give good spectra both in the wet and in the dried state, for films which give good spectra while the cells are wet usually give very poor diffraction patterns when dry. In the case of the goat and of *Hemitragus* it is difficult to demonstrate shrinkage even by photographic methods, for the limit of resolution amounts to about 10 p.c. of the major axis of the cell. Emmons has selected the cells of the former animal for the one photographic measurement which he records, although by this selection the difficulties are placed at a maximum. He states that the result of this measurement is to show that the goat cell is smaller in plasma than it is in the dried state, but does not support the

finding either by details or by figures. We presume, however, since our technique is stated to have been followed, that the difference observed was greater than 0·2μ, and, as the difference was also observed by the eriometer, that it was of the order of 0·4μ at least. Even were we not disposed to dispute this result, we are unable to see that it is compatible with Emmons' own statement that the diameter of the dried cell is a dimension which is a very close approximation to the corresponding dimension of the cell as it exists in the circulating blood, for the finding appears to us to lead to exactly the opposite conclusion.

Using the eriometer as a means of measuring the diameter of dried cells, Emmons arrives at certain far-reaching conclusions regarding the relation of hæmoglobin content to surface area of the circulating cell and of the red cell count to red cell diameter. It is not our purpose to discuss these relations at this time, but it will be understood that in so far as they rest upon eriometer measurements, the conclusions stand disputed.

Summary.

1. A modification of the diffraction method for measuring the diameter of erythrocytes is described. This modification makes use of monochromatic light derived from the individual lines of the mercury arc.

2. It is shown that the formula deduced for the position of the diffraction bands produced by a haphazard distribution of cells is applicable when monochromatic light is used.

3. This method detects the change in diameter of erythrocytes on drying.

The expenses of this investigation were in part defrayed by a grant from the Government Grants Committee of the Royal Society. Our especial thanks are due to the New York Zoological Society for enabling us to obtain the blood of *Hemitragus jemlaicus*.

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A STUDY OF THE ELECTRICAL FIELD SURROUNDING SKELETAL MUSCLE[1].

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(*From the Cardiac Department, University College Hospital Medical School.*)

Part I. The problem of action current.

A strip of muscle is taken from the body, suspended in air and stimulated at one end; two contacts are placed in line upon the muscle, at a distance from the stimulating electrodes, and are connected to a sensitive galvanometer. As is well known from the experience of many workers, the electrical effect recorded is a diphasic curve of which the first phase indicates a potential at the proximal contact lower than that at the distal contact, the proximal contact being that first reached by the excitation wave.

In a previous investigation (1) of cardiac muscle, the tissue strip was examined not suspended in air but deeply immersed in a quantity of saline. The electrical currents developed about excitable tissue so immersed seem never to have been investigated; the need for so proceeding has arisen out of the method of electrocardiography in which the muscle from which records are taken always lies deeply buried in a conducting medium. Using immersed heart muscle strips and stimulating and leading off as above, a simple diphasic effect was never obtained. The curves were always polyphasic, and the initial deflection invariably indicated relative positivity of the proximal contact. On the basis of these and related observations views were expressed in regard to the manner in which currents arise and flow in the medium surrounding immersed cardiac muscle. It was considered that the field of potential due to such currents could best be explained on the basis of doublets; and this hypothesis was fully discussed.

From further consideration it seemed that the records obtained from immersed cardiac muscle might conceivably be peculiar to that type of muscle, composed as it is of a syncytium of extremely short individual fibres. It seemed important therefore to investigate the action currents

[1] Work undertaken on behalf of the Medical Research Council.

of some simple long parallel-fibred skeletal muscle, similarly immersed, in order to ascertain whether there is any essential difference in the manner in which action currents arise from the two tissues in question.

Galvanometric responses have accordingly been obtained from two leads applied to skeletal muscle under the following conditions: (*a*) when the saline surrounding the tissue is limited, as for example when a muscle is kept moist and is suspended in air; (*b*) when the saline surrounding the tissue is in considerable amount, *e.g.* when the muscle is either immersed in, or lies in contact with the surface of a large quantity of saline; (*c*) when two contacts are on injured and uninjured regions respectively of a partly injured muscle, and the latter is suspended (i) in air, (ii) in a considerable body of saline; and (*d*) when one contact is at the surface of the tissue, and the other at some distant point in a considerable quantity of saline surrounding the tissue. These variations in leading off were chosen for reasons that developed as the investigation proceeded.

The responses recorded are a continuous expression of the differences of the potentials of the regions in the saline to which the leads are applied; these differences of potential are in turn an expression of the lines of current flow that result from muscle activity. Any hypothesis put forward to express these responses in terms of specific levels of potential at specific regions of the active tissue must, clearly, explain all recorded curves with equal facility provided the conditions of their inscription are understood.

Method.

The five experiments that follow have certain features in common.

Preparations used. The sartorius of the frog was selected as it is composed of long parallel fibres. Nerve conduction was eliminated by preliminary curarisation. This was obtained by injecting 0·1 to 0·2 c.c. of a 1 p.c. solution of curare into the dorsal lymph sac of the decerebrate animal some 20 minutes before exposing the muscle. Curarisation was considered complete when powerful electrical stimuli applied direct to the vertebral column produced no response in the muscles of the lower limbs.

Electrodes. The stimulating electrodes consisted of two parallel silver wire needles connected to an adjustable induction coil. They were placed in contact with one end of the tissue in such a way that the plane passing through them was perpendicular to the long axis of the muscle. The recording contacts were composed of glass tubing of 3 mm. bore drawn

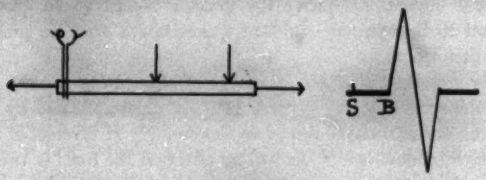
to a point of 1 mm. in diameter or less. Polarisation was diminished by filling the contact end with 12 mm. of gelatin made up with Ringer's solution. Above this was placed 5 cm. of saturated copper sulphate solution into which dipped pure copper foil.

Interpretation of curves. The electrical responses were recorded by means of a string galvanometer. The string used had a diameter of 1·5μ and a length of 6 cm. Its period of vibration was of the order of 0·002 sec. with a resistance in the circuit of 14,000 ohms, and a tension such that the shadow, distant 1 metre, was deflected through 1 cm. by a potential difference of 30–50 millivolts. The recorded curves were interpreted with due regard to the limitations of the string. The tension of the latter was, whenever possible, so adjusted that its speed was sufficient to record accurately the changing potential differences between the leading off contacts. Certain of the experiments were conducted at room temperature; in such circumstances the degree of tension required to effect accuracy of the complete curve is not obtainable without reducing the dimensions of the graphic record beyond convenient limits; in consequence, such records obtained at room temperature are used only to indicate the direction of the initial deflection. In curves obtained at temperatures below 12° C. the rate at which the deflections are inscribed is much slower than in uncooled preparations. In these curves the measured properties of the string, maintained at a tension yielding conspicuous deflections, prove the graphic record to be qualitatively exact, and to be without sufficient quantitative error to produce any material distortion.

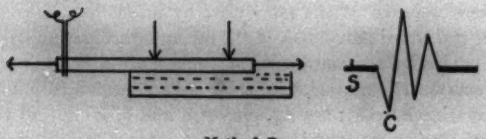
To test recorded responses for escape of stimulating current. Security that the deflections of any given curves are uncomplicated by escape of stimulating current was obtained as follows. (1) If reversal of the direction of the stimulating current had no effect on the form of the recorded response, the latter was regarded as containing no elements of the stimulus. (2) In certain experiments the stimulus and the muscle response were recorded on the same plate by means of a second galvanometer. If the initial movement of the string recording the stimulus was unaccompanied by a simultaneous movement of the string connected to the contacts on the muscle, the latter were regarded as unaffected by the stimulating current; see *e.g.* curves *a* and *b*, Pl. I. All the curves used for analysis were examined in one or other of the above ways, and complication by stimulating current definitely excluded.

Exp. I. To study the string galvanometer record obtained by placing two contacts on a curarised sartorius stimulated directly at one end, (A) when the muscle is suspended in air, (B) when both leads are on a portion of muscle in contact with, or surrounded by, a considerable quantity of conducting medium.

The sartorius is removed from a curarised frog by carefully dividing the connective tissue adhesions between it and underlying muscles. The tendinous and muscular insertions into tibia and pelvic girdle respectively are divided close to the bone. It is suspended in air with its normally subcutaneous surface uppermost by means of two threads tied to its ends. These are attached to two upright supports and the tension of the strip adjusted so that direct stimulation results in an approximately isometric contraction. Stimulating electrodes are placed in contact with the pelvic end. Two recording contacts are fixed resting lightly on the uppermost surface of the muscle, one near the middle and the other towards the tibial end. Fig. 1, method *A*, shows this arrangement of muscle and electrodes. The stimulus from the coil is so adjusted that conspicuous contractions occur at break, and feeble or no responses at make. The tissue is kept just moist by occasional washing with Ringer's solution at room temperature. Curve *a*, Pl. I, shows one example of the typical diphasic action current responses obtained from a series of sartorii thus examined.



Method *A*.



Method *B*.

Fig. 1. Showing the arrangement of the muscle, electrodes and dish of saline in methods *A* and *B*; and also the typical resultant curves when the muscle responds to single shocks.

A flat dish filled with saline is next brought up under the muscle so that, without in any other way disturbing the preparation, about two-thirds of the tissue are brought into contact with the fluid in the dish, while one-third of the tissue from the point of stimulation remains in air. Fig. 1, method *B*, shows this arrangement of muscle and electrodes. The proximal contact is at least 6 mm. from the edge of the dish. Responses are recorded from single stimuli precisely similar to those previously used.

Curve *b*, Pl. I, is typical of the responses obtained in several such experiments. Curve *a* shows a diphasic variation in the potential difference of the two contacts. The first phase represents relative negativity of the proximal contact, the second phase relative negativity of the distal contact. Curve *b*, on the other hand, shows an initial deflection below the base line, revealing a relative positivity of the proximal contact[1]. This is followed by a deflection above the base line indicating relative negativity of the near contact. The remaining deflections vary in form in different experiments and will be discussed later.

We may conclude from these curves that whenever the muscle is suspended in air and surrounded by an extremely limited layer of conducting medium, the electrical record of the response to a single shock is typically diphasic. When, however, the surrounding conducting medium is increased to considerable dimensions, an initial relative positivity of the proximal lead is invariably displayed.

In further support of this conclusion may be described briefly the original experiment of which method *B* is a simple modification. An isolated sartorius is suspended vertically in a vessel containing saline at 10° C., the pelvic third remaining in air. It is stimulated at the upper end and led off by two contacts applied to the surface of the completely immersed section. The galvanometric responses are always of the general type shown in curves *c* and *d*, Pl. I. The response is always polyphasic and an initial relative positivity of the proximal contact is invariably recorded. The number of phases present depends on the distance separating the leads. Curve *c* was obtained when the leads were 6 mm. apart, and curve *d* when 17 mm. apart.

We may next similarly examine curves obtained from muscle injured beneath one contact, a method well known to yield *monophasic curves* from muscle suspended in air.

[1] For the initial positive deflection to be well marked it is essential that the proximal contact should be an appreciable distance from the edge of the trough.

Exp. II. To examine the string galvanometer record obtained from a partly injured curarised sartorius by means of one contact on an uninjured and another on an injured region of the tissue (A) when the muscle is suspended in air, (B) when the regions led off are associated with an extensive conducting medium.

An isolated sartorius, curarised as before, is partly injured by dipping the distal fourth into saline at 55° C. It is suspended in air by threads attached to the ends at a tension sufficient to render contraction approximately isometric. Two leading off electrodes are placed on injured and uninjured regions respectively. The tissue is kept moist by occasional washing with Ringer's solution. It is stimulated at the uninjured pelvic end. Curve *e*, Pl. I, shows a record of the action current following a single stimulus obtained in such an experiment. The response is approximately monophasic in character, there being some overshoot at the termination of the deflection.

A flat dish containing saline is now brought up beneath the muscle so that both regions led off are in contact with the surface of the saline. Special care is taken to ensure that the electrodes and tissue remain otherwise undisturbed. About 1 cm. of the tissue from the region of stimulation is allowed to remain in air, as in Fig. 1, method *B*. Curve *f* is one example of the action current response now obtained. Such curves invariably show an initial positive phase.

From these observations we may conclude that a monophasic curve is obtained when the partly injured tissue is suspended in a very limited conducting envelope. The presence of a considerable body of saline in the region of both contacts, however, produces an initial relative positivity of the proximal lead.

It has thus been shown that immersed skeletal and cardiac muscle behave similarly in an important respect, namely, that when a wave of excitation passes beneath two contacts in turn, the graphic record displays an initial deflection representing a relative positivity of the lead first encountered. It remains therefore to determine whether the current flow round skeletal tissue is to be explained on a basis similar to that put forward for cardiac muscle in the previous investigation.

In any investigation of the source of action currents from excited tissues there is one difficulty that must constantly arise in connection with the interpretation of graphic records. So long as both leading off contacts are in close relation with the active tissue, their potentials will be simultaneously influenced; and it will be a practical impossibility to analyse the graphic record of their potential difference in terms of their

individual levels. Injury beneath one contact has been advocated as meeting this difficulty; there is, however, no obvious reason why such a procedure should ensure fixation of the level of potential at the injured surface. As will be shown later, there is indeed every reason to suppose that activity of uninjured tissue in the neighbourhood of the injury does materially affect the level of potential at the injured surface.

The ideal method clearly is one in which changes in potential at the surface of active tissue can be measured relative to some unvarying standard. Such a method would disclose those regions at which the level of potential is at a maximum, indicating the presence of sources or anodes, and other regions simultaneously at a minimum, disclosing the situation of sinks or kathodes.

The method that suggests itself as nearly meeting the ideal requirements is to immerse the active tissue in a large body of saline and place one electrode near the tissue and the other at a distance. This distance should be sufficient to eliminate any material change in the potential at the distant electrode in consequence of tissue activity.

Exp. III. To study the changes in potential occurring at points close to the surface of an uninjured curarised sartorius immersed in a considerable saline medium, and directly stimulated at one end.

Method C. The sartorius of a curarised frog is carefully removed by dividing the connective tissue binding it to the neighbouring structures of the thigh. The tibial tendon and the muscular pelvic ends of the muscle are cut close to their attachments to bone. It is suspended in air between two upright supports by threads attached to the ends, and its tension adjusted until contraction following direct stimulation is isometric. A glass dish filled with saline is brought up beneath the muscle so that the tibial half is in contact with the surface of the saline, while the pelvic half is suspended in air. The naturally subcutaneous surface is arranged to remain uppermost. One contact is placed at any distant point in the saline; the other is fixed resting lightly on the surface of the immersed half. Stimulation is applied at the pelvic end, and responses following single stimuli recorded. Curve *g*, Pl. II, is typical of responses obtained by this method.

To eliminate so far as possible the effects of dissection injuries on the excitation response, it was thought desirable to repeat the experiment in a modified form.

Method D. The thigh of a curarised frog is skinned and placed on a large pad of cotton wool saturated with Ringer's solution. The distal

half of the sartorius is carefully dissected away by dividing its connective tissue attachments to the limb. It is gently stretched and suspended in air by means of a thread attached to its tibial tendon. Additional pads of saturated wool are laid round the preparation, until the attached half of the muscle is freely bathed in saline, while the distal freed portion remains exposed in air. Stimulating electrodes are applied to the muscle close to the tibial end. Leading off contacts are arranged, one anywhere on the pad at a distance, the other resting lightly on the surface of the sartorius in its uninjured proximal half. Single break stimuli of sufficient strength to evoke a good contraction are imparted to the tissue. The tension of the muscle is adjusted to render contraction practically isometric. In this method there is a minimum of disturbance in the region of the muscle led off. Curve *h*, Pl. I, was obtained in such an experiment. It is typical of responses recorded by this method.

The curves obtained by these two methods resemble each other closely. They consistently show first a positive effect, corresponding to an elevation of the potential at the near contact. This is followed by an equally definite negative effect, *i.e.*, a fall in potential below the level present previous to excitation. The remaining deflections, as previously stated, are difficult to analyse in view of the tendency of the string to oscillate when returning from the wide negative excursion. It was consequently decided to cool the muscle in the hope that a slowing down of the electromotive mechanisms would facilitate accurate analysis of the final deflections of the recorded response.

Preparations were set up in precisely the same way as described under methods *C* and *D* above, except that all saline employed was previously cooled down to the region of 6° C. The tissue was allowed to rest in the cooled saline for some time previous to stimulation. Curves *i* and *j*, Pl. II, are examples of the responses now seen. They are triphasic in form, with a definite positive end phase.

Discussion and a possible explanation of the recorded electrical responses of active skeletal muscle.

We are faced with the necessity of having to explain on a common basis three characteristic responses from uninjured tissue. They are: (i) a diphasic curve from two contacts on tissue suspended in air; (ii) a polyphasic curve with an initial positive deflection from two contacts on tissue suspended in a considerable body of saline; and (iii) a triphasic effect including two positive phases from immersed tissue with only one direct contact.

In attempting to explain these recorded responses, it must be borne in mind that the current flow in the saline external to the tissue is alone under consideration. This qualification is to be emphasised since all our observations refer only to the difference of potential between two regions in the external medium.

On theoretical grounds it may be assumed that when current flows in a limited homogeneous medium, such as the body of saline surrounding the muscle strips in the foregoing experiments, there must be at least one region at the boundary at which it enters, and at least one other at which it leaves. For brevity we will refer to two such regions simultaneously present as a doublet. The region at which current enters the medium will be referred to as an anode, and that at which it leaves as a kathode.

If now we can explain on a common basis involving doublets all responses recorded under known conditions we shall have postulated an hypothesis experimentally supported and theoretically acceptable.

It was previously shown(1) that the field of potential in the saline surrounding immersed cardiac muscle can be explained on the basis of doublets in the following way. As a wave of excitation passes down the tissue it is supposed to be accompanied by a wave or succession of doublets, each of which is so arranged that the positive pole is a short distance ahead of the negative pole; that is, the region in which the current enters the external medium from the tissue is in front of the region at which it re-enters the tissue, the term "front" indicating the direction in which the wave is travelling. Doublets thus oriented are referred to as primary doublets. Subsequently there is supposed to follow a second succession of simultaneous regions of current entry and exit at the tissue surface, but oriented reversely, that is, the region of exit is now ahead of that of entry. The latter doublets are referred to as secondary doublets.

We will consider first the galvanometric response which is to be expected from two contacts on an air surrounded strip of moist muscle; it is the moisture on the tissue that constitutes the external medium the current flow in which is under consideration. Let us consider the conditions obtaining at the instant at which the excitation wave has reached the middle of the strip, and confine ourselves for the moment to the theoretical effect we should expect were the wave of excitation to give rise to a single primary doublet as postulated above, and situated near the middle of the strip. Current will flow through the saline envelope from the anode to the kathode, through all regions of the envelope in

which flow can take place. If the layer of moisture be excessively thin, it is clear that current arising at the anode cannot flow towards the corresponding end of the strip, for if it does so it has to return by the same path eventually to reach the kathode; and current cannot flow in the same path in opposite directions at once without being reduced to zero.

This is equivalent to saying that provided the saline envelope is sufficiently limited, all regions between either pole of the doublet and the corresponding end of the strip are isopotential, for if in a conductor there is no flow of current between two points the potentials at those points are equal. That is, the potential in the saline between the anode and the corresponding end of the strip is at a constant level; and the potential between the kathode and the end of the strip corresponding to it is also at a constant level, the latter being lower than the former.

Under these circumstances two contacts leading to a galvanometer and placed either between the anode and the corresponding end of the muscle, or between the kathode and the end corresponding to it, will yield no deflection. If, however, the two contacts be arranged one on either side of the doublet, the galvanometer will immediately record a current flowing from the contact nearer the anode towards that nearer the kathode. Hence, if the saline be sufficiently limited, current will flow through the galvanometer from two contacts on a strip only when one or both poles of the doublet are situated between them; and it will flow in only one direction, namely from the contact on the same side as the anode towards the other.

If therefore we postulate a sufficiently limited layer of saline, and a succession of similarly oriented doublets to pass down the strip, two contacts will yield a monophasic curve indicating a relative negativity of the contact first reached. Similarly, a succession of doublets of reversed polarity passing down a strip similarly led off will yield a monophasic response in the reverse direction, *i.e.* the contact first encountered will be relatively positive.

In practice a strip of skeletal muscle under these conditions yields a diphasic curve, the phases of which are qualitatively similar to those just described. We may thus regard the diphasic response obtained from a strip of moist tissue suspended in air to be readily explained on the basis of two sets of primary and secondary doublets as postulated above.

We will now consider the field of potential in a large volume of saline due to a doublet at the surface of immersed tissue. It is easy to show experimentally, and is readily understood theoretically, that any region

nearer the anode than the kathode is at a higher potential than any other region nearer the kathode than the anode; also that as we approach the anode from any region on the anode side of a plane surface perpendicularly bisecting the line joining anode and kathode, the potential steadily rises. This is equivalent to saying that the potential in the surrounding medium is at a maximum at the anode and at a minimum at the kathode. As the primary doublets approach the proximal of two contacts on a strip of immersed muscle, the potential at this contact therefore steadily rises above that at the far lead. We would thus expect, on the basis of the doublet view, just such an initial positivity as has been seen in all curves recorded from immersed strips.

We come next to the curve we would expect from an immersed strip with one direct and one distant lead. It follows from the above description of the field of potential round a doublet at the surface of immersed tissue, that as the primary doublets approach the direct contact, its potential is gradually raised to a maximum; it falls to a minimum immediately after the wave of primary doublets has passed. The primary doublets alone would thus produce a diphasic curve with an initial positive phase. Similarly, a wave of secondary doublets alone would produce a diphasic curve with an initial negative phase.

If the effects of the first and second waves are sufficiently separated in time, we would expect at most four deflections in the order $+ -, - +$. The positive sign here represents positivity of the near electrode relative to the potential of the distant lead, and *vice versa*. It is manifest that if the secondary doublet follows the primary doublet sufficiently quickly, its field of relatively low potential may fuse with that of the primary, and so yield a triphasic curve as the whole effect. The same result would happen if, as is conceivably the case, our tight string of $1{\cdot}5\mu$ diameter, quick and sensitive as it is, fails to record one deflection completely before the next begins. The fact that triphasic and not tetraphasic curves are actually recorded is not surprising therefore, and is not opposed to the hypothesis put forward.

Further tests of the doublet view may be carried out as follows.

Test I. Referring to Exp. I it may be recalled that two leading off contacts were maintained in a fixed relation to a curarised sartorius. Responses were recorded from the tissue (*A*) when suspended in air, and (*B*) when in contact with a considerable body of saline. Fig. 1 shows the two arrangements along with tracings of typical responses. According to the doublet postulate there is in method *A* no differential effect on the potentials at the two electrodes until the wave of excitation has

passed the proximal contact. This then becomes relatively negative. In method *B*, on the contrary, the potential at the proximal contact becomes increasingly positive as the excitatory process approaches it. Its potential changes abruptly to relative negativity when the wave of excitation has passed beyond it. Hence, apart from the lag of the recording instrument, we would theoretically expect the interval between the stimulus and the beginning of the deflection in method *A* to agree closely with the interval between the stimulus and the peak of the initial positive deflection in method *B*[1]. Table I shows the measurements of these intervals in a series of six experiments. Four different muscles were employed, the contacts being shifted and the experiment repeated in the case of two.

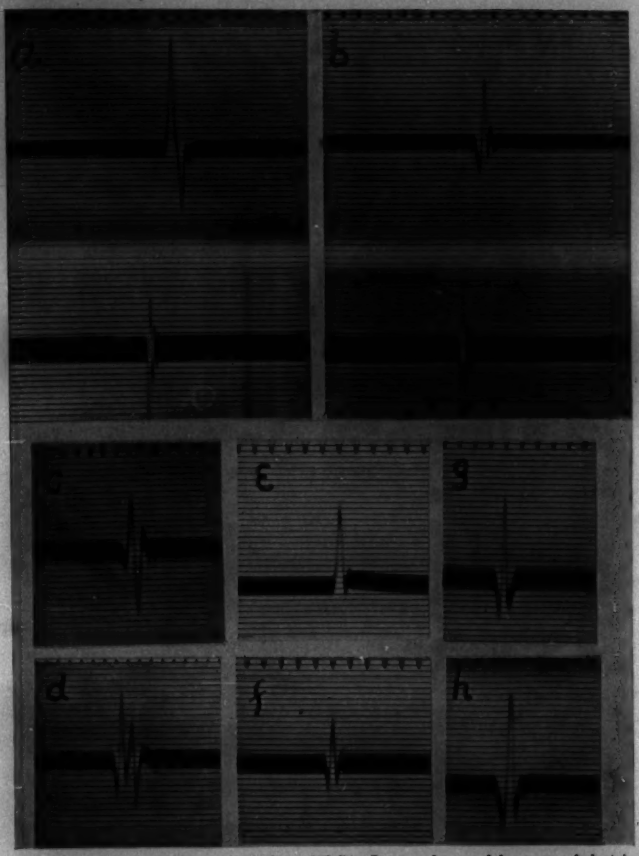
TABLE I.

	Muscle in air	Muscle on conducting medium
	Time in secs. between stimulus and beginning of relative negativity of proximal electrode	Time in secs. between stimulus and peak of initial relative positivity of proximal electrode
Preparation	*S B*	*S C*
1	0·0074	0·0080
2	0·0054	0·0055
3	0·0059	0·0060
4	0·0043	0·0046
5	0·0057	0·0058
6	0·0061	0·0063

There is a slight excess in favour of the response derived from the partly immersed tissue. This excess is well within the error of measurement. The agreement is, indeed, sufficiently close to conclude that the experimental findings bear out the theoretical expectations referred to above.

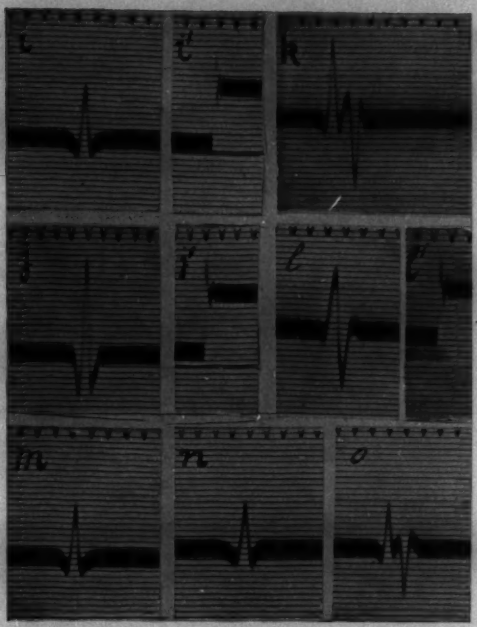
Test II. As shown in Exp. III, one near and one distant contact in relation with an immersed strip yield a graphic record triphasic in form. It follows, on the basis of previous reasoning, that two contacts on the same strip should yield a response which, when analysed, should be composed of two superimposed triphasic effects. The second of these should be an approximate mirror image of the first. In the event of the two contacts being sufficiently far apart, we might expect all six phases to be seen in the record in the order $+ - +, - + -$. This distance will depend on the velocity of the wave of excitation, the time relations of

[1] In practice, however, since the string is moving from rest in method *A* and is reversing its direction of movement in describing the positive peak in method *B*, we would expect a measurement of the actual curve to show a slight excess in favour of those derived in method *B*.



Records at room temperature (about 17° C.). In records *a* and *b* curves of electrical response of muscle (above) and signal of stimulation (below) are shown. The diphasic curve (*a*) obtained from the unimmersed muscle is altered by immersion of the muscle, an initial positive deflection appearing in record *b*. Records *c* and *d*, two polyphasic records from immersed muscle, the pair of leading-off contacts being 6 mm. apart in *c* and 17 mm. apart in *d*. Records *e* and *f*, records from a muscle injured under one contact, immersed and unimmersed, respectively.

Records *g* and *h*, triphasic curves taken from immersed muscles with one contact on the muscle and the other at a distant point in the surrounding saline (methods *C* and *D*).



Records from muscle immersed in saline at about 6° C. Records *i* and *j* were taken in the same way as were records *g* and *h*; records *i'* and *j'* show the corresponding deflection times of the string. Records *k* and *l*, showing the effects of bringing the two leading-off contacts close together (*l*); *l'* exhibits the corresponding deflection time of the string. Records *m*, *n* and *o* were obtained from the three leads represented by *AD*, *BD* and *AB*, respectively, in Fig. 2.

In this and the preceding plate, the time is marked by 1/100 sec. tuning fork; the ordinates are 20 millivolts = 1 cm.; resistance of electrode-galvanometer circuit 10,000 ohms.

the doublets involved, and the strength, or potential difference, of the latter. The conditions must be such that the entire galvanometric consequences of excitation are over in the region of the first contact before they materially affect the potential at the other. The following experiment was accordingly carried out.

Exp. IV. To study the string galvanometer record of the response obtained from a curarised sartorius immersed in chilled saline and led off by two contacts separated as far as possible.

The sartorius of a bull frog is set up precisely as in Exp. III, method *C*. Two leading-off contacts are placed on the uninjured surface of the muscle, one 3 mm. from the edge of the dish, and the other about 17 mm. nearer the distal end. The electrical responses from isometric contractions are recorded, and curve *k*, Pl. II, shows one example of the type of response seen. The distal electrode is then moved to within 6 mm. of the proximal contact. Curve *l* shows the type of response now obtained. Curve *k* distinctly shows six phases in the order named above. In view of the measured properties of the string, these phases probably represent with fair accuracy the relative potentials of the two contacts during the course of excitation. Curve *l*, inscribed when the contacts were moved closer together, shows a curve of only four phases, indicating the fusion of six phases into four that has occurred as the result of bringing the leads closer together. The presence of six phases in the response from immersed skeletal muscle led off by two contacts is a striking phenomenon, when we consider that it was theoretically anticipated on the basis of what appeared to be a rational explanation of the typical diphasic curve from a strip suspended in air. Test II thus offers further evidence in support of the doublet view.

That the response from two contacts on an immersed curarised sartorius is composed of the interference of two opposite triphasic effects originating at the near and far contacts respectively may be further demonstrated as follows.

An isolated sartorius from a curarised bull frog is again set up as in Exp. III, method *C*. The arrangement of saline dish and suspended tissue is represented in Fig. 2. *A* and *B* are two leading-off contacts resting on the muscle about 25 mm. apart, and *D* is a third contact fixed at any distant region of the saline. The responses obtained from single stimuli when leads *AD*, *BD* and *AB* are employed are shown in Pl. II, curves *m*, *n* and *o* respectively. Clearly the six-phased response *o* is an algebraic summation of two curves, namely, the triphasic response *m*

and a mirror image of the triphasic response *n*. If leads *A* and *B* are placed sufficiently close together, the interference of their potentials will result in a four-phased response, as was seen in curves *c* and *l*.

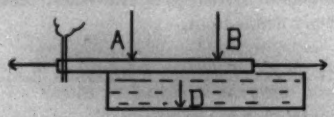


Fig. 2. The arrangement of muscle, saline bath and electrodes to illustrate leads *AD*, *BD* and *AB*, described in the text.

It has thus been shown that two contacts on a strip of muscle may yield responses which are two-phased (curve *a*), four-phased (curves *c* and *l*), or six-phased (curves *d*, *k* and *o*), depending upon the volume of the saline surrounding the tissue, and upon the distance separating the leading-off contacts. It has also been shown that these very different responses are in strict accord with the doublet postulate.

Part II. The problem of injury current.

It is known that the potential at the surface of the injured region of a partly injured muscle is lower than that at the uninjured surface of the same muscle. Whether the potential at the latter changes at all, and how the change, if any, is related to the external surroundings appear at present to be unknown. This will now be examined.

The problem of the origin of injury current has to be approached from two distinct points of view: (i) we must ascertain the lines of current flow in the conducting medium outside the tissue boundary, *i.e.* in the external circuit; and (ii) we must relate these lines of flow to specific electromotive mechanisms localised within or at the surface boundary of the tissue itself. If the latter mechanisms are known the lines of flow in the external circuit may be deduced, provided the nature and configuration of the external medium be known; the converse is, however, not necessarily true. It might be possible to deduce the seat of the E.M.F.'s of injured tissue from a knowledge of the lines of flow of injury current, if the tissue were sufficiently simple in structure to render its phase interfaces few in number and obvious in situation. For in any system of fluid conductors electromotive forces may be said to occur only across the interfaces or phase boundaries separating two different homogeneous media.

The problem thus naturally falls under two headings: (*a*) the field

of potential in the medium surrounding the tissue; (*b*) the mechanism and sites of the electromotive forces concerned in its production. Since the present investigation aims solely at the interpretation of galvanometric records in terms of the electrical events in the medium external to the tissue boundary, the problem of injury current will be approached experimentally from the former standpoint only.

In studying the lines of current flow in any homogeneous medium, the problem may be said to be solved when all the regions of current entry and withdrawal at the boundary are known; that is, when all anodes and kathodes have been localised.

The sites of origin of positive and negative ions respectively at the surface of an injured muscle are clearly independent of the quantity of the saline surrounding it. The latter can influence only the course and the amount of current flowing per unit time.

To locate the regions at which current arises and disappears at the surface of a partly injured muscle, we may usefully employ the device adopted in the previous section and seek the maxima and minima of potential at the tissue boundary relative to an approximately unchanged level at a region distant in the fluid surrounding the preparation. Thus, the injured tissue is bathed in a large volume of saline, one lead is fixed at a distance, and an exploring electrode is moved from point to point at the surface of the tissue and the difference of potential noted.

Preliminary note on method. To investigate the field round any given tissue the latter is either totally or partly immersed in a bath of Ringer's saline sufficient in extent to permit one electrode to be fixed some 7 to 10 cm. from the tissue. The other electrode, to be referred to as the exploring electrode, is mounted on an easily adjustable carrier permitting ready movement of the electrode from one region to another, its exact position being read on a scale specially arranged. The electrode-galvanometer circuit is temporarily completed at will by closing a suitably inserted contact key, the contact being maintained sufficiently long for the galvanometer deflection to be noted. In order to eliminate electromotive forces arising within the electrode-galvanometer system, sufficient compensatory current is introduced to ensure a zero reading with both contacts close together at a distance from the tissue, immediately before moving the exploring contact to the neighbourhood of the preparation. The difference in the potentials at the ends of the two electrodes is estimated in the usual way by comparing the deflection with that given by a standard potential difference. For reasons depending on the general coarseness of any injured preparation, there is

no point in attempting to record potential differences arising from injured tissues more accurately than can be done in the above simple way. In order to make the galvanometer readings approximately equivalent to the actual potential differences existing in the external medium, it is essential that the resistance of the electrode-galvanometer circuit should be as high as is commensurate with adequate sensitivity. In the experiments to be described the external resistance was of the order of 10,000 ohms.

Exp. V. To explore the electrical field in the neighbourhood of an injured muscle.

Two different arrangements of injured tissue and saline bath will be described in order to demonstrate the constancy of the experimental findings.

Method I. The skin is removed from the hind leg of a frog and the muscle to be used, whether sartorius or gastrocnemius, is left *in situ* on the limb. The exposed limb is placed upon a large pad of saturated cotton wool which is so arranged that the upper surface of the muscle to be examined appears above the level of the surrounding saline, and is therefore separated from air by only a limited conducting envelope. The exploring electrode is allowed to rest in this limited layer barely touching the muscle. Preliminary exploration over the uninjured muscle is first carried out to observe the extremes of potential difference between distant and near contacts before gross injury is inflicted. If the skin is removed with care it is possible to obtain a preparation practically free from injury currents; and provided preliminary measurements reveal potential differences of not more than a millivolt or two between the distant and the near contact, unavoidable dissection injuries may be regarded as too small to interfere with, or mask, the very conspicuous differences observed after gross injury. Transverse injuries are then made in the selected muscle by snipping cleanly with sharp scissors or by a single brief contact with a heated needle. The exploring electrode is now moved a millimetre at a time along the uninjured surface towards the injury, and the deflection noted for each position. The results are shown in Graph (*a*), Fig. 3.

Method II. Single muscles are removed as carefully as possible and totally immersed in a saline bath, or, as in the previous method, are placed upon a cotton wool pad sufficiently saturated to keep the tissue covered by a thin layer of fluid. Removal of the entire muscle involves relatively gross injury at distal and proximal insertions, and along the

under surface where the muscle is attached to the limb by nervous, vascular and fibrous structures. In the case of the sartorius, injury at

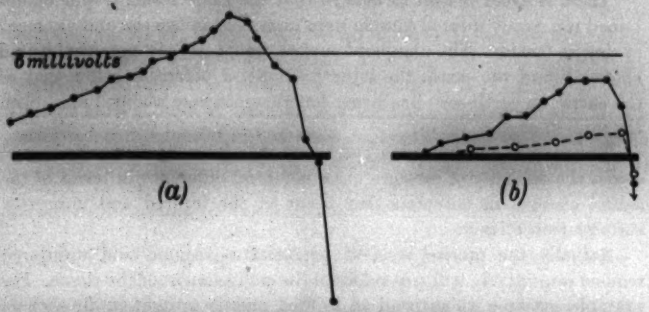


Fig. 3. A muscle injured at its right-hand end is represented by the heavy black line. The graph represents the potentials found at corresponding points of the surface. Potentials positive to a distant point are plotted above, and negative below.

(*a*) a 28 mm. muscle examined *in situ*;
(*b*) a 21 mm. muscle removed from the limb.

the tibial end may be minimised by careful detachment of the tibial tendon; complete transverse division of the muscle through its pelvic third provides the injury that gives rise to the field. When completely immersed the muscle may be suspended from a suitable frame by threads attached to the ends. Exploration is carried out by keeping one contact fixed at a distant region of the saline and moving the exploring contact 1 mm. at a time along a line running parallel to the long axis of the muscle and in contact with its surface.

Graph (*b*), Fig. 3, pictures the results of an experiment carried out in this way. The line joining the small black circles represents the potential difference at the surface when the muscle was placed upon a moist pad; the line joining the white circles, the potential difference when the same tissue was completely immersed in a large bath and suspended by threads tied to its ends. These two curves from the same tissue are qualitatively similar, and differ only in degree.

Comment on Exp. V. The main feature of interest that emerges from this experiment is that, relative to the level of potential at the distant fixed contact, the potential at the uninjured surface of the injured muscle becomes increasingly positive as we approach the injured region, falls to isopotential at the region approximately 2 mm. from the edge

of the visible injury, and then rapidly falls to a conspicuous negativity when the actual injured surface is reached.

There is every reason to believe that the same result would be obtained if a partly injured muscle were immersed in the sea and explored in similar fashion. The uninjured surface would display potential raised above that of the earth, the injured surface a potential below that of the earth, an earth-zero line being found somewhere about 2 mm. from the injured edge.

The method of immersing the tissue in a large field of saline overcomes the difficulty of interpreting a potential difference in terms of the actual changes in potential that occur at the injured and uninjured surfaces respectively.

Actually the precise level of potential at injured and uninjured regions respectively will depend upon the environment of the tissue. For example, suppose we suspend an injured muscle upright in air with its injured surface just dipping into a large bowl of saline. If we explore the surface of the muscle as before by means of one electrode at a distance in the saline, and an exploring contact moved from point to point on the tissue, relatively large positive values will be found on the air-surrounded portion of the uninjured surface, and relatively small negative values, if any, at the injured end. Suppose we reverse the muscle so that the uninjured end is dipping into the saline, and again explore its surface. Large negative values will now be found on the injured region and small positive values, if any, at the immersed uninjured surface.

Now since the saline in the bowl remains at a more or less constant level of potential, we must conclude from the above findings that the actual potentials at the surface of the tissue vary with the position of the tissue relative to the saline; that is to say, the actual potentials at the surface are modified by the environment of the tissue, while the current flows through the external medium between regions which are determined only by the site of the injury.

This point is stressed to emphasise the need of avoiding descriptions of injury and action currents in terms of variable quantities whose values in any given case depend on the conditions of the external medium. When the muscle current is described in terms of the regions at which the current enters and leaves the tissue, the phenomenon is described in terms which specify activities completely independent of the conditions of the external medium.

An approximate but clear impression of the lines of current flow

round an injured muscle may be gained from the following simple experiment. It is based on the principle that in any conductor the current flows in a direction which is perpendicular to the equipotential surfaces. Suppose, therefore, that we reduce the conducting medium surrounding the tissue to an approximate plane, and map out experimentally the equipotential lines, it is possible to obtain a fairly good idea of the actual lines of current flow by drawing in continuous lines which will cut the measured equipotential loci at right angles. A saline-saturated circular piece of filter paper forms an excellent "plane" conductor and an isolated sartorius divided near its attachment to the pelvic bone a suitable preparation for the purpose.

Exp. VI. To map out the equipotential surfaces in a plane circular field surrounding a sartorius injured at one end.

Method. A circular filter paper of diameter about 18 cm. is divided into small numbered squares by ruled pencil lines in order to facilitate localisation of any particular point on its surface. It is placed on a plane horizontal wax surface and saturated with Ringer's solution. The tendinous attachment of a frog's sartorius is freed from the tibial bone, and the entire muscle carefully dissected away from its attachments to the underlying structures; a transverse section in its upper third severs it from its proximal end. It is placed upon the filter paper so that its long axis passes over the centre of the paper, with its injured surface about 2 mm. away. Fig. 4 shows the arrangement of tissue and filter paper.

AE and *CJ* represent two perpendicular diameters specially included when squaring the filter paper; the long axis of the tissue is made to lie along *AE*. Two pointed electrodes are placed upon the paper; they are connected to a sensitive galvanometer through a special contrivance permitting compensation of electromotive forces arising within the galvanometer-electrode circuit. One electrode is kept fixed at a selected site, and the other is moved from place to place on the moist field until the deflection in the galvanometer disappears. The two regions are therefore isopotential, and the position of the exploring contact is noted. In this way a number of "spots" on the moist field may be localised which are at the same potential as the point of application of the fixed contact. The line joining these spots is roughly an equipotential surface at the potential of the fixed contact. The fixed contact is then moved to another selected site and its "equipotential locus" similarly obtained.

Fig. 4 shows the result of one such experiment. Sites *A*, *B*, *C*, *D*, *E*, *F*, *G*, *H* and *I* were selected for the fixed contact, the lines passing

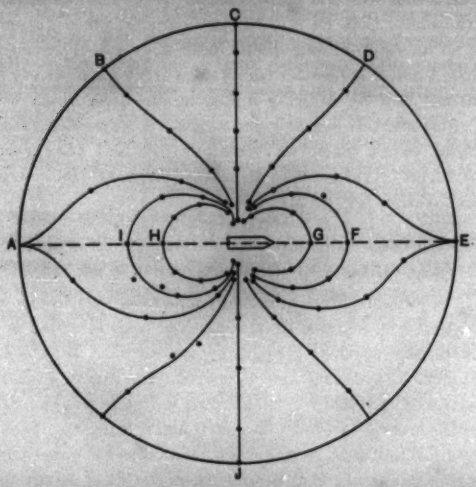


Fig. 4. A chart of the equipotential lines in a plane circular field surrounding an injured sartorius muscle. For comparison with this is Fig. 5, a similar chart of the equipotential lines surrounding two pointed electrodes joined to a battery.

through the black circles representing their respective equipotential loci. When the moving contact approaches the tissue, small changes in its position produce great variations in the deflection of the galvanometer, and accurate localisation of the equipotential spot is not practicable.

If we now measure the potential difference between the straight equipotential *CJ* passing through the muscle near the injury, and each of the other equipotentials shown, it immediately becomes evident that the field of potential is peculiarly symmetrical. That is, if we regard the straight equipotential line as at an arbitrary level of *O* then all regions to the left (*i.e.* in the direction of the injured surface) are *O* − and all regions to the right are *O* +.

Measurement of the potential difference between equipotential *CJ* and the equipotential passing through *B* = − 13 units; through *D* = + 14 units; through *A* = − 16 units; through *E* = + 17 units; through *I* = − 20 units; through *F* = + 22 units; through *H* = − 25 units; and

through $G = +28$ units, revealing the degree of symmetry to which reference has been made.

If the field were exactly symmetrical about the diameter *CJ* it would mean that we could describe the tissue as behaving precisely as though the current flow were arising from a "point" anode and a "point" kathode situated symmetrically near the centre on the diameter perpendicular to *CJ*, the anode being on the side of the uninjured surface and the kathode on the side of the injury. That is, in the language employed in the previous section of this paper, and from the point of view of the external field of current flow, the muscle would behave precisely as though it were replaceable by an equivalent doublet.

The similarity between the current flow round an injured sartorius and the flow round an artificial doublet may be demonstrated in the following way. A saturated circular piece of filter paper is prepared as before. Two pointed electrodes joined to a battery are placed on the diameter *XY*, Fig. 5, equidistant about 3 mm. from the centre on either

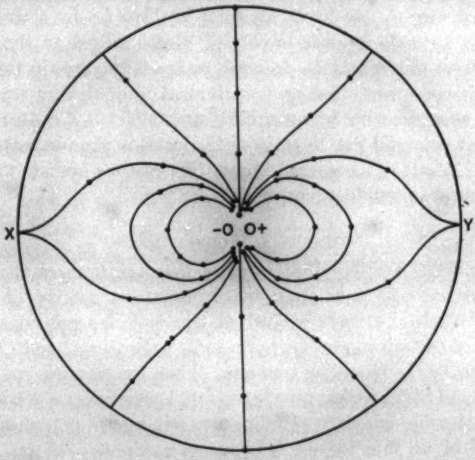


Fig. 5.

side of it. A make and break contact key is inserted near the battery so that current flow may be controlled and unnecessary polarisation avoided. The same points are chosen as in Exp. VI for the position of

the fixed contact, and equipotential loci ascertained as before. Fig. 5 shows the result of one such experiment. Comparison of the equipotential lines in Figs. 4 and 5 reveals how very similar the two fields are.

The question arises whether the results of these experiments on injured sartorii afford any evidence by means of which we may localise the sources of origin of negative and positive ions respectively at the surface of an injured muscle. It is not possible to specify how much of the injured surface is the source of negative ions, and how much of the uninjured surface is the source of positive ions; but the field of potential revealed by these methods suggests strongly that the main source of positive ions is from the uninjured surface over a region situated comparatively near the edge of the visible injury.

Certain useful deductions may be based on the evidence presented by Exp. VI. Histologically the sartorius is composed of a number of separate long parallel fibres, each of which in the above circumstances has an injured and an uninjured surface. The aggregate of injured fibres has been shown to give rise to an external field of flow which can be reproduced very closely by an artificial doublet, *i.e.* by a simultaneous anode and kathode in close proximity, and localised at the region of injury. From this it may be deduced, so far as the flow in the external medium is concerned, that each individual injured fibre may also be regarded as replaceable by an equivalent doublet; or, conversely, that the field of potential due to an injured muscle is the summation of the individual effects of its injured fibres. The reasons leading up to these conclusions are given in an appendix.

Summary and conclusions.

Experiments are described to show that the form of the galvanometric response obtained from two contacts on a strip of curarised uninjured skeletal muscle excited at one end depends upon (1) the quantity of the saline medium surrounding the tissue; and (2) the distance apart of the recording contacts. When the tissue is suspended in air the record is the well-known type of diphasic curve; when suspended in a considerable quantity of saline the response is polyphasic, and is composed of an interference between two triphasic effects occurring successively at each of the leading-off contacts. It is shown that during the passage of a single wave of excitation down the strip the potential at any arbitrary point on the surface first rises above, then falls below, and subsequently again rises above the level of potential obtaining at the surface during the resting state.

When the tissue is injured beneath the distal of the two recording contacts, the record of the action current is monophasic only when the strip is suspended in air; it shows an initial deflection disclosing a relative positivity of the proximal contact when the partly injured strip is immersed in saline.

The conclusion is drawn that electric currents flowing round active skeletal muscle arise in precisely the same manner as was previously found to be the case in cardiac muscle, the difference being merely one of degree.

The manner in which action currents are supposed to arise may be briefly described as follows. When a wave of excitation passes down a strip of long-fibred skeletal muscle, the flow of current in the saline surrounding it may be explained on the assumption that the initial electrical effect of excitation of each small fraction of an excited fibre is to produce a simultaneous anode and kathode at the surface of the tissue, with the anode in the direction in which the wave is travelling. This simultaneous anode and kathode (or doublet) is followed shortly afterwards by a second electrical effect also of the nature of a simultaneous anode and kathode, of which however the anode is now in the direction from which the wave has come. That is to say, in terms of potential theory, the excitatory process is accompanied by a three-fold change in the level of potential at the surface of the muscle, namely, first a rise (positivity), then a fall (negativity), and finally a further rise (positivity), the terms positivity and negativity being employed in relation to the level of potential obtaining at the surface of the quiescent tissue.

On this view, it is clear that the potential at the surface of the excited area at a certain instant of time is indeed negative to that of the saline at all other regions of the tissue surface, and in this sense it is correct to speak of the "negativity of the active region." Such an expression, however, is one readily leading to misconception. Misconception arises when development of the "negativity" is regarded as the sole or chief change that occurs at the surface of active tissue, when actually the change that occurs is three-fold and in the order "positivity," "negativity," "positivity."

Experiments are described in which it is shown that the current round a resting injured sartorius arises somewhere on the uninjured surface and disappears somewhere on the injured surface. When the muscle is immersed in a large volume of saline, the potential of the saline at the injured region is conspicuously negative, and that at the

uninjured surface quite definitely, though less conspicuously, positive to the level of potential at distant regions of the surrounding medium. Further, this positivity reaches a maximum at a region a short distance from the edge of the injury.

It is shown further that under certain conditions the current flow round an injured sartorius is very similar to that which occurs when the muscle is replaced by an artificial anode and kathode situated at the region of injury. From this it is deduced that so far as the current in the surrounding saline is concerned, an injured muscle behaves very much as though replaceable by an equivalent simultaneous anode and kathode (or doublet). From this and supplementary arguments it follows (*a*) that a single injured fibre may also be regarded as replaceable by an equivalent doublet, and (*b*) that the field of potential round an injured muscle is the summation of the individual contributions of the injured fibres.

I desire to acknowledge my indebtedness to Sir Thomas Lewis and Professor A. V. Hill for their kindly help and criticism throughout this investigation.

REFERENCE.

1. Craib. Heart, 14. p. 71. 1927.

APPENDIX.

The distribution of potential and the current flow, in a conducting field around a doublet of the type considered in the preceding paper, is a well-known mathematical problem, of which the solution must satisfy Laplace's equation and the boundary conditions characteristic of the system. The problem cannot in general be solved, but whether it can or not we may imagine the potential at any point P due to a doublet at the point A to be represented by some mathematical function $V_{P,A}$, where V depends upon the coordinates of P and of A, and on the strength and direction of the doublet at A.

Imagine for example that we are interested in the potential difference which will arise between two different points P and Q when a number of similar injured sartorii are arbitrarily arranged at the points $A, B, C,$ etc. The potential at P due to the first is $V_{P,A}$, at Q it is $V_{Q,A}$, so that the difference of potential arising from the first sartorius alone is $V_{P,A} - V_{Q,A}$. Potentials, however, are additive, so that if we imagine

the injured muscles to be placed simultaneously at the different points A, B, C, ... the total potential difference between P and Q is

$$V_{P,A} + V_{P,B} + V_{P,C} + \ldots - V_{Q,A} - V_{Q,B} - V_{Q,C} - \ldots.$$

To take a simple application of this result, two identical injured sartorii side by side, facing in the same direction, will produce twice the potential difference between any two distant points in the conducting field surrounding them: three identical injured sartorii, side by side, similarly will produce three times the potential difference of one alone, and so on. Conversely, the field of potential produced by an injured parallel-fibred muscle is twice the field produced by half the muscle, three times the field produced by one-third of the muscle, and so on. Hence, if the muscle be composed of n individual injured fibres the field of the whole muscle is n times the field due to a single injured fibre. Again, two parallel identical injured sartorii placed near to one another and facing in opposite directions will neutralise each other's potential effects at distant points, so that no potential difference will exist between such points. These deductions can be verified experimentally.

Thus we see that the potential at any arbitrary point in a conducting medium surrounding an active tissue is related directly to the potential at all regions of origin of positive and negative ions respectively. Moreover, the potential at P is the sum of the potentials produced at P by anodes and kathodes individually. The problem therefore of the distribution of potential, or current, around an active or injured tissue is one which can be expressed in simple mathematical terms, although its actual solution may in any given case be outside our reach.

THE EFFECT OF EXPERIMENTAL ANÆMIA ON THE SIZE OF THE SPLEEN.

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Under diseased condition, a change in the size of the spleen is often found to be associated with a change of hæmoglobin content in the blood. The same is also true in laboratory findings. One would naturally be curious as to what is the relation of this organ to the change of oxygen carrying power of blood. Cruickshank(1), examining the hæmoglobin content of the spleen, considered it as a reservoir of blood. Barcroft and Stephens(2) came to the same conclusion. Abderhalden and Roski(3) found no increase of erythrocytes in the peripheral circulation with administration of adrenaline, chloralhydrate, or ether after splenectomy. Binet, Cardot and Williamson(4) showed definite increase of red blood corpuscles in the peripheral circulation with asphyxia in normal but not in splenectomised animals. Strohl, Binet and Fournier(5) and also Nitzescu and Cosma(6) demonstrated the same phenomenon in condition as anoxemia. They contended that through its contraction the spleen furnishes this extra amount of hæmoglobin. With all the foregoing evidence, it seemed probable that the spleen might contract to small size in cases of anæmia so as to play its important rôle as a regulator to the oxygen carrying power of the blood.

The present investigation is to study the response of the spleen towards anæmia. Observations, therefore, were made directly on the spleen in artificially produced anæmia while the function of the hæmatopoietic system is not disturbed. The experiments are more or less acute in nature and the production of prolonged blood change is not attempted.

Rabbits are used for these experiments. Although a large series of guinea-pigs has been tried, they never survive long after operation.

Method.

The spleen of the experimental animal is exteriorised by using the extracutaneous technique of Barcroft and Stephens(2). In rabbits, the wound is generally healed in less than two weeks. During this time,

the "spleen area" undergoes a preliminary enlargement and then gradually reduces to a steady size. The growth of the skin epithelium is rather rapid and the entire organ is covered in about four to six weeks. At post-mortem examination, the skin is found to have grown over the spleen and there is an amount of fibrous tissue around it. When the spleen is fully under cover of the epithelium, dressing is no longer necessary. The animals rarely bite their spleens any more; occasional injuries may however be produced by scratches.

The size of the spleen is estimated by measuring its outline traced on a piece of transparent oil-silk. After the organ becomes subcutaneous, the edges can always be readily felt and marked with ink for tracing. During the days when the spleen is partially under cover of the skin, the outline is rather difficult to map out and the measurements on those days are thereby not quite exact. But throughout the experimental period, the control animals are observed in the same manner for comparison. After the epithelium grows completely over, the size of the spleen always appears larger than that of the few previous days. The reason for this is not clear.

For slow bleeding, the blood is allowed to run from a cut into the ear-vein directly into a measuring cylinder. An amount of 20 to 30 c.c. is usually collected in less than half an hour. Intravenous injections are always given through the lateral ear-veins as soon as the spleens are measured after bleeding. In all of the experiments hæmoglobin is determined in a Haldane hæmoglobinometer.

Experiments.

Several animals have been bled by about one-tenth of the total blood volume at a time. With repeated bleedings the spleen shrinks to a surprisingly small size. Several days after cessation of the bleedings it regains and maintains its size. Since bleeding has long been known to cause contraction of the spleen—a fact which has lately been verified (2, 7, 8), it is unnecessary here to do more than say that what has been found in other animals is true also in rabbits. One experiment, therefore, as shown in Fig. 1, is sufficient for illustration.

Another way of producing anæmia is by subcutaneous injection of phenylhydrazine as was done by Morawitz (9) and many other previous workers. The dosage given to the rabbits in these experiments is 10 mg. per kilo body weight and the injection is repeated every two days. Shortly after the first injection the colour of the spleen becomes a dusky blue; this colour persists for several days after the last injection. The

blue colour is most likely due to the formation of methæmoglobin. But if so, the size of the spleen does not confirm the change due to methæmoglobin as found by Frey(10).

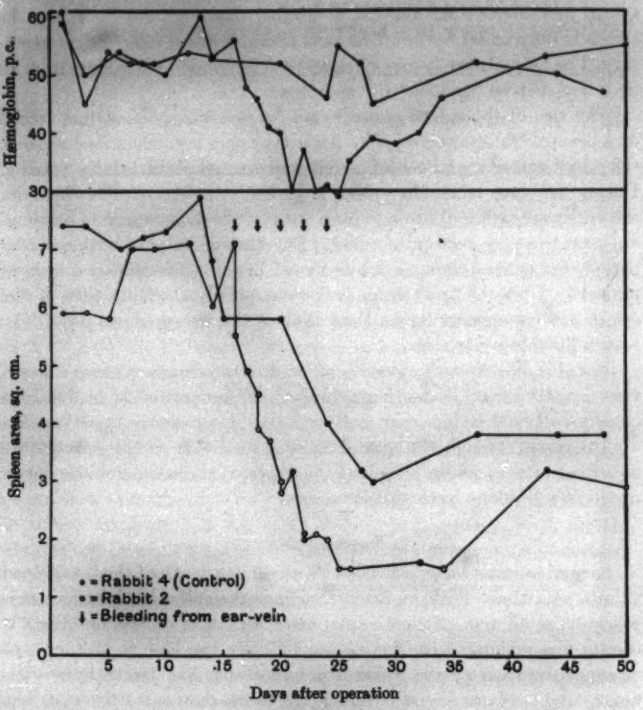

Fig. 1.

Throughout the period of observation, the "spleen area" does not change in any constant sense, in spite of the progressive diminution of the hæmoglobin content in the peripheral circulation. One animal died as a result of the injections while others made an uneventful recovery. As seen in Fig. 2, the contour of the curves follows that of the control quite closely. Only the one which died during the course of injections

showed that the spleen has a tendency to become large during the last few days.

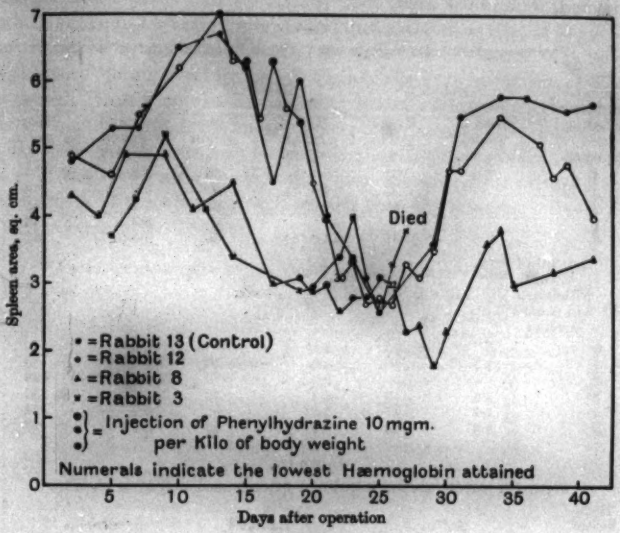
Spleen area, sq. cm.
Died
• =Rabbit 13 (Control)
• =Rabbit 12
▲ =Rabbit 8
▪ =Rabbit 3
= Injection of Phenylhydrazine 10 mgm. per Kilo of body weight
Numerals indicate the lowest Hæmoglobin attained
Days after operation

Fig. 2.

Hæmolysis produced by intravenous injections of sterile distilled-water has also been tried. But the results are not as successful as those of other workers who have made similar injections of far larger quantities (9). The animals in these experiments died either instantaneously or within a few hours after the injections. Only one animal survived two injections of 15 and 20 c.c., and died with a third one (the fifth day after the first injection). In this animal, the hæmoglobin was reduced from 65 to 52 p.c. and the size of the spleen showed hardly any change at all.

In order to produce anæmia without altering the blood volume, the animals are bled and immediately equal quantities of heparinised plasma are injected. This plasma has been previously obtained from other animals, the serum of which has been tested to show no agglutination with the red blood corpuscles of the recipients. The spleens, of course, become small with bleeding; but as soon as the amounts of plasma are injected, they return to or even become larger than the original sizes. These experiments,

as shown in Table I, indicate the change of the spleen quite clearly even when a small amount of blood is let out and a similar amount of plasma injected.

TABLE I.

(Figures in parentheses indicate size of spleen immediately after bleeding.)

Days before and after bleeding and injection	Rabbit 2		Rabbit 12		Rabbit 13	
	Size of spleen sq.cm.	Hæmoglobin %	Size of spleen sq.cm.	Hæmoglobin %	Size of spleen sq.cm.	Hæmoglobin %
4	3·9	53	5·1	58	5·2	55
3	—	—	4·6	60	5·2	63
2	3·6	53	4·8	65	5·4	63
1	—	—	—	—	5·3	58
0	4·0	51	4·0 (3·3)	66	5·5 (3·7)	63
Amount of blood withdrawn and plasma injected	20 c.c.		7·5 c.c.		25 c.c.	
0	5·7	40	3·8	—	5·3	—
1	4·5	40	3·9	59	5·6	45
2	—	—	3·9	70	5·6	46
3	3·8	40	4·1	62	5·5	50
4	4·1	47	4·2	62	5·7	54
5	3·8	48	3·9	63	5·0	50

TABLE II.

(Figures in parentheses indicate size of spleen immediately after bleeding.)

Days before and after bleeding and injection	Rabbit 2		Rabbit 4		Rabbit 15	
	Size of spleen sq.cm.	Hæmoglobin %	Size of spleen sq.cm.	Hæmoglobin %	Size of spleen sq.cm.	Hæmoglobin %
4	—	—	5·7	52	5·9	61
3	4·1	47	5·9	52	5·9	58
2	3·8	48	6·0	52	6·1	53
1	4·1	48	5·8	52	6·4	53
0	3·8 (2·9)	48	5·7 (3·7)	53	6·5 (4·5)	56
Amount of blood withdrawn and cell suspensions injected	20 c.c.		7·5 c.c.		20 c.c. (bled) 25 c.c. (inj.)	
0	2·9	—	4·2	—	4·8	—
1	3·0	48	4·3	54	6·0	60
2	3·6	52	4·5	56	5·4	56
3	3·6	49	4·8	60	5·6	62
4	3·7	51	5·0	62	5·6	62

Table II shows the effect of reducing the plasma without producing anæmia: that is plasmaphæresis (10). In these experiments the animals are bled and then the cells suspended in salt solution are injected equal

to the volume of cells taken out. The red blood corpuscles, which show no agglutination with serum of these animals, are obtained by killing other rabbits. These cells are washed three times and then diluted with sterile saline to the same hæmoglobin concentration as that of the blood of the experimental animals. After the injection of the cell suspension, the spleen does not return to its original size until several days later, although there is no anæmia present. In one experiment (rabbit 15) a larger quantity of cell suspension is purposely injected after bleeding; so that there is a definite high hæmoglobin content in the blood. But the size of the spleen after the injection, even though comparatively larger than those of the others, is still smaller than the original.

TABLE III.

(Figures in parentheses indicate size of spleen immediately after bleeding.)

Days before and after bleeding and injection	Rabbit 4		Rabbit 13	
	Size of spleen sq.cm.	Hæmoglobin %	Size of spleen sq.cm.	Hæmoglobin %
3	4·9	62	4·8	52
2	5·2	55	4·7	55
1	5·3	64	5·0	57
0	5·7 (4·2)	58	5·0 (3·3)	60
Amount of blood withdrawn and saline injected	25 c.c.		25 c.c.	
0	4·3	—	3·2	—
1 hr.	4·8	48	3·2	—
1	4·5	48	3·0	42
2	4·6	52	3·2	46
3	4·7	52	3·8	48

Two control experiments are then performed by substituting sterile saline for plasma or cell suspension. After the injection, the spleen, though it may temporarily become larger than its size after bleeding, resumes the small size in a short time. The results are shown in Table III.

CONCLUSION AND SUMMARY.

(1) The exteriorised spleens of rabbits, like those of other animals, get smaller gradually in the weeks following exteriorisation. If, however, the rabbits are bled from time to time the spleen shrinks much more rapidly in the bled animals than in the controls and if the bleeding be discontinued, the spleen enlarges to the size of the control.

(2) Anæmia produced by various other methods does not produce an appreciable effect to the size of the spleen.

(3) Plasmaphæresis or saline transfusion does not cause much enlargement of the spleen after its size has been reduced by bleeding.

(4) So in bleeding, the reduction in the size of the spleen is due to ischæmia rather than anæmia.

I wish to acknowledge my indebtedness to Prof. Barcroft who has kindly permitted me to work in his laboratory and has shown the keenest interest throughout. Thanks are also due to Dr Anrep whose suggestions and criticism were extremely valuable to this work.

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THE DISCHARGE OF IMPULSES IN MOTOR NERVE FIBRES. Part I. Impulses in single fibres of the phrenic nerve.

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It is generally agreed that the motor nerve fibres perform their function in the body by conducting impulses of the normal type, but there is little agreement as to the way in which these impulses are discharged. According to one line of argument a powerful contraction is brought about by more or less synchronous volleys of impulses occurring at the rate of 50–80 a sec. in each motor neurone; according to another the rate of discharge is very much higher and the apparent synchronisation revealed by the electromyogram is caused by the properties of the recording instrument or of the muscle. What happens with a relatively weak contraction is too uncertain even to have become a subject for controversy. In the present investigation we have obtained direct records of the discharge in individual motor nerve fibres innervating the diaphragm. We cannot assume that our results can be applied to every kind of reflex contraction, but for this particular case they seem so definite that it is unnecessary to set out the whole of the evidence which has been brought forward by indirect methods in the past[1].

The phrenic is an ideal nerve for investigations of the activity of the motor centres, for the respiratory discharge needs no external stimulus to maintain it, its intensity can be varied by controlling the air supply to the lungs and it occurs at definite intervals separated by periods of inactivity. The electric responses in the whole nerve trunk have been recorded by Dittler and Garten (3) with the string galvanometer, and by Gasser and Newcomer (4) with the same instrument used in conjunction with a valve amplifier. Their records (made from the dog) show that in each period of inspiration the electric response takes the form of a series of oscillations at the rate of about 70 a sec. Each oscillation resembles

[1] This evidence has been summarised elsewhere by one of us (1) and various points have been discussed by Forbes and his co-workers (2).

the typical mono- or diphasic wave of action potential such as would be produced by stimulating the nerve electrically. If the resemblance were complete it would follow that each oscillation is due to a synchronous volley of impulses in many fibres, but more perfect recording instruments show that the oscillations are in fact considerably longer and more complex. A lack of complete synchronisation between the different neurones would account for this, but there are other possibilities which have been put forward (Fulton(5)), and a study of the composite response of the entire nerve trunk is not likely to give decisive evidence however perfect our recording system may be. To be sure of what is happening in the single nerve fibre we have to devise a method which will put out of action all the other fibres in the nerve. The recording of impulses in a single fibre presents no difficulty, but the problem of isolating the fibre seemed much more formidable. Fortunately this has turned out not nearly so difficult as we had imagined.

Method.

Most of our experiments have been made on rabbits anæsthetised with urethane. The nerve we have used is the uppermost root of the phrenic, a slender branch arising from the third cervical nerve and running down over the scalenus anterior muscle for about 15 mm. before it is joined by the larger branch from the fourth cervical. Its motor fibres supply the anterior third of the diaphragm. Sections stained with osmic acid show that there are about 150 fibres in the nerve, all of the large medullated type with the exception of five or six very small medullated fibres. The nerve is easily dissected out in the neck and cutting it causes no obvious disturbance of respiration, since the greater part of the phrenic is left intact.

For diminishing the number of active fibres we have adopted the simple method of dissecting the nerve with fine needles under a binocular microscope. It is cut distally at its junction with the branch from the fourth cervical and laid in a pool of warm Ringer on a small platform of glass coated with black paint on its under surface. The platform is held rigidly by a rod fixed to an upright at the side of the board on which the animal rests. A Zeiss binocular dissecting microscope is focussed upon the nerve, which is illuminated from above by an electric lamp.

The first step in the dissection consists in splitting the connective tissue sheath which surrounds the nerve fibres. The sheath is pulled out on either side leaving the exposed nerve in the middle as in Fig. 1 *A*. The nerve is then split longitudinally in such a way as to leave a small strand of intact fibres in the middle (Fig. 1 *B*). The lateral bundles are cut

across by pressure with a fine hook (Fig. 1 *C*) and the central strand is then split in the same way. The object of the dissection is to leave a few

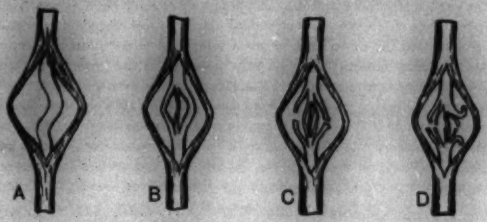

Fig. 1. Method of dissecting nerve so as to divide all but two or three fibres.

fibres in the middle of the nerve intact but separated as far as possible from one another. If this is accomplished, the remaining fibres are cautiously divided until only the smallest strand is left joining the two ends of the nerve (Fig. 1 *D*). We have rarely attempted to carry the dissection to the stage at which only one fibre is left undivided. One fibre chosen at random might turn out to be afferent and not motor, and it is much harder to isolate a single fibre than to isolate a group of three or four. The electrical records usually provide sufficient evidence of the number of motor fibres in action, and the actual number of undivided fibres can be checked by microscopical examination after the experiment is over. The number of undivided fibres has always been greater than the number of motor fibres which appear to be in action in our records.

The process of dissection and still more the subsequent manipulation of the nerve might be expected to prove rapidly fatal to the few fibres which bridge the gap, but fortunately the connective tissue sheath (which has been split but not cut transversely) acts as a most effective splint and prevents undue tension or displacement. In fact the nerve can be handled as though it were still undamaged, and the surviving fibres give a discharge which shows no signs of alteration for periods as long as two hours.

Each stage in the dissection is controlled by observing the impulse discharge in the nerve distal to the division. The distal portion is slung on two small paint brush electrodes (cf. Adrian and Zotterman(6)), the proximal connected to earth and the distal to the input of the three valve amplifier described elsewhere(7). The amplified action currents can be photographed with the capillary electrometer, but until the final stages are reached it is usually more convenient to lead them to a telephone or

loud speaker and estimate the character of the discharge by the ear instead of the eye.

The use of the telephone for investigating nerve or muscle action currents is well known, for the telephone has much the same electrical sensitivity as the string galvanometer. We have been accustomed for some time to use a telephone connection in the output of the amplifier system before making photographic records. In the present work, however, in addition to the telephone we have found it a great convenience to have a continual sound record of the nerve discharge made by a loud speaker. This has a separate two valve transformer-coupled amplifier of its own and it is connected to the output of the main amplifier through a ·01 mf. condenser. As the connection to the electrometer is through a 1 or 10 mf. condenser and a 120,000 ohm resistance the loud speaker connection introduces practically no distortion nor reduction in amplitude in the electrometer record. The arrangement of the connections is shown in Fig. 2. When only a few fibres are in action the electrometer

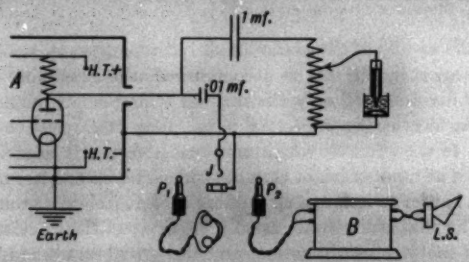

Fig. 2. Connections for telephone or loud speaker used simultaneously with capillary electrometer. The output of the main amplifier *A* (last stage only shown in figure) is connected through a ·01 mf. condenser to the jack *J*. The plug P_1 leads to a telephone and P_2 gives an alternative connection to the loud speaker *L.S.* through the second amplifier *B*.

excursions may be too small to detect on a screen, but they produce a series of faint clicks in the loud speaker, and it is thus possible to control the dissection, to expose a plate at the moment when the discharge is at its height, etc., without the inconvenience of wearing telephones.

When photographic records are to be taken the animal is placed on an insulated platform inside an earthed metal box, to avoid a slight ripple from alternating current mains which often appears when the preparation is unshielded.

Results.

(*a*) *The discharge of the entire nerve.*

A large number of records have been made in different animals before the division of the nerve fibres. In normal quiet breathing, which occurs at a rate of about 55 a minute in the rabbit, each discharge lasts about

$\frac{1}{2}$ sec. and consists of an irregular and rapid succession of potential changes. There is sometimes an indication of large excursions recurring at a frequency of about 20–30 a second, but it is often difficult to make out any kind of regularity. When forcible inspiratory movements are produced by clamping the air tubes to the trachea, each period of contraction may last as long as a second and the rate of respiration slows down. The character of the discharge changes and it becomes much more like the phrenic discharge in the dog as recorded by Gasser and Newcomer[1]. Instead of the irregular succession of potential changes there appears a more or less orderly sequence of large waves at frequencies ranging from 50–90 a sec. The degree of regularity varies from one animal to another, but it is always more pronounced with forcible breathing. The discharges shown in Fig. 3 are fairly typical, and records from the highest root of the

Fig. 3. Action current record of 3rd cervical root of phrenic, all nerve fibres intact. *Above*: normal breathing (discharge lasts somewhat longer than usual). *Below*: air tubes clamped. Discharge shows large waves with a greater approach to regularity. Time marker gives ·125 sec. intervals.

phrenic in the decerebrate cat show much the same change. It may be noted here that the electromyogram of a voluntary contraction has the same tendency to develop regular waves at 50–70 a sec. (the Piper rhythm) when the contraction is forcible and prolonged.

[1] In Gasser and Newcomer's experiments the thorax was opened and the lungs were inflated artificially, though there is no reason to suppose that the respiratory discharge was more intense than it would be in normal breathing.

(b) The discharge of individual fibres.

The effect of the reduction in the number of active fibres can be seen in Fig. 4 (Exp. 14). The records are from plates, all of which were exposed

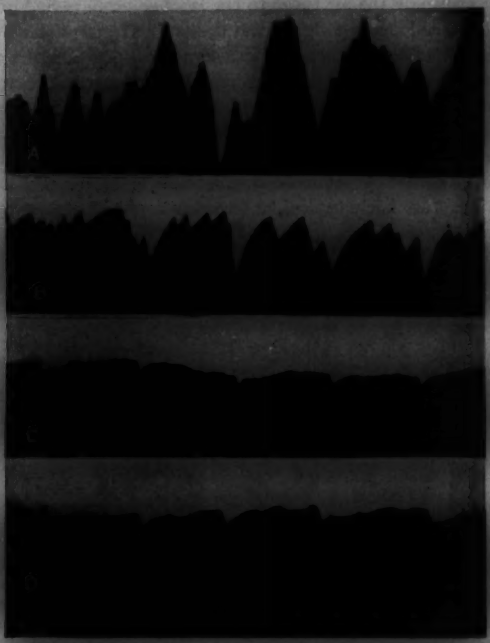

Fig. 4. Action currents at different stages of dissection. Exp. 14. Normal breathing. *A*. Nerve intact. Electrometer one-fourth maximum sensitivity. *B*. About nine-tenths of the fibres divided. Maximum sensitivity. *C*. All but three fibres divided, but probably only one in action. The impulses form a single series at a frequency of 40 a sec. *D*. Another record made some minutes later. Time marker gives ·01 sec. intervals.

during the period of inspiration but not necessarily at the same time after the beginning of the discharge. In *A* both electrodes are on the proximal, undivided part of the nerve and the electrometer is shunted to one-fourth of its normal sensitivity. The record shows the complex structure of the large excursions in quiet breathing. In *B* about nine-tenths of the nerve

fibres have been cut, the electrometer is working at its maximum sensitivity and the individual impulses can be seen. At intervals of about ·025 sec. several impulses coalesce into larger excursions. In *C* still more of the fibres were divided and the record shows a single series of impulses recurring at intervals of ·023–·025 sec. *D* is another record where the impulses are not spaced quite so regularly.

The nerve was examined after the experiment was over and it was found that the gap was bridged by three fibres. Two of these showed no sign of damage but the third was badly contorted. Of the two fibres which appeared to be undamaged it is unlikely that more than one is concerned in the discharges shown in Fig. 4 *C* and *D*, for the impulses appear to form a more or less regular series and never follow one another at less than ·02 sec. Records on bromide paper covering the entire period of inspiration show that almost all the impulses occur in a single series, though there is a suggestion of two impulses at a much closer interval at

Fig. 5. Exp. 13. Final stage of dissection. *A* and *B*. Normal breathing. Frequency of impulses 27 a sec. *C* and *D*. Air tubes clamped. Frequency 68 a sec. in *C* and 55 in *D*.

the very beginning of the discharge. It is possible therefore that the second fibre transmits an occasional impulse, though it may well have been an afferent fibre from the end organs in the muscle.

The discharges in Fig. 4 were all obtained during unobstructed breathing. In this animal cutting off the air supply gave a discharge in the intact nerve which was longer but not much larger or more regular than with normal breathing and the force of the movements was not much increased. Records from the nerve below the divided area during asphyxia show some increase in the frequency of the impulses, but the change is only from an average of 37 to an average of 50 a sec.

The effect of clamping the air tubes is shown more clearly in Figs. 5, 6 and 7 (Exps. 13 and 19). In both of these preparations the gap in the

Fig. 6.

Fig. 7.

Fig. 6. Exp. 19. Final stage. *A*. Normal breathing. Frequency of impulses 28 a sec. *B*. Air tubes clamped. Frequency 50 a sec.

Fig. 7. Exp. 19. Final stage. Discharge throughout the period of inspiration. *A*. Normal breathing. *B*. Air tubes clamped. Time marker gives ·125 sec. intervals.

nerve was bridged by four or five undivided fibres, but the discharge has the same character as in Exp. 14, *i.e.* the impulses appear to form a single series. With normal breathing the frequency of impulse discharge is slower than in Exp. 14 (about 27 a sec.) and the increase on asphyxia is greater, the frequency rising to 68 a sec. in Fig. 5 *C*. Fig. 7 gives the discharge during the entire period of inspiration in Exp. 19 and shows the increased duration in asphyxia as well as the increased frequency.

The records in Figs. 4–7 are typical of a large number obtained from these three experiments and from two others where the dissection was

TABLE I.

Frequency of discharge in preparations giving a single series of impulses. Data obtained from continuous records of several periods of inspiration.

	Normal breathing		Air tubes clamped	
	Maximum frequency per sec.	Average frequency per sec.	Maximum frequency per sec.	Average frequency per sec.
Exp. 12	18	15	82	40
	23	19	60	38
	19	15	68	50
Exp. 13 (*a*)	26	23	72	43
	29	21	80	56
	28	25	80	60
(*b*)	24	21	86	51
	25	21	104	72
	26	23	112	78
Exp. 14	48	35	56	46
	48	38	60	50
	48	31	50	51
Exp. 17	24	22	42	27
	24	20	42	30
Exp. 18	24	18	64	36
	26	20	44	35
Exp. 19	27	25	50	37
	28	26	50	43
	28	25	60	47

TABLE II.

Duration of interval between successive impulses in "single fibre discharges," throughout the period of inspiration.

Exp. 13.

Normal breathing. Intervals (secs.): ·049, ·045, ·038, ·032, ·028, ·029, ·028, ·027, ·028, ·030, ·027, ·028, ·073.

Air tubes clamped: ·072, ·056, ·040, ·032, ·028, ·028, ·025, ·021, ·023, ·023, ·017, ·020, ·021, ·021, ·016, ·022, ·016, ·017, ·020, ·022, ·016, ·017, ·016, ·024, ·022, ·019, ·014, ·027, ·024, ·112.

Exp. 19.

Normal breathing: ·056, ·054, ·040, ·044, ·038, ·034, ·040, ·039, ·052.

Air tubes clamped: ·032, ·032, ·036, ·033, ·032, ·030, ·032, ·025, ·025, ·025, ·028, ·030, ·024, ·028, ·024, ·025, ·024, ·024, ·025, ·024, ·025, ·025, ·024, ·023, ·023, ·026, ·024.

successfully carried to the stage at which a single series of impulses appeared. The data as to frequency and regularity of the impulses are given in Tables I and II which are constructed from continuous records of the type shown in Fig. 7. It will be seen that all five experiments agree very closely with one another and this is perhaps the best proof that they show the normal discharge of a single nerve fibre. In every case we are dealing with a nerve containing several undivided fibres (the actual number varying from three to six or more), but a further reduction in the number of fibres has always left us with one or two apparently intact but with no impulse discharge of any kind. In fact we have never found any intermediate stage between the complete absence of a discharge and one of the type shown in Figs. 4–7. For this reason it is extremely unlikely that such discharges are produced by two or more fibres acting in rotation, and we may accept them as due to a single fibre. As examples of the type of discharge due to several fibres we may take Fig. 8 (Exps. 12 and 22).

Fig. 8. Grouping of impulses in preparations with several intact fibres. *Above*: Exp. 16. Two fibres in action. *Below*: Exp. 32. Four fibres in action.

In both of these there were five or more undivided fibres and the impulses are obviously grouped.

The uniformity in the "single fibre" records makes it unlikely that they are the product of nerve fibres which have been so damaged as to affect their power of conduction, and this is supported by the fact that the character of the discharge does not change appreciably in the course of an hour or more.

There are, however, two points in which there is a departure from normal conditions. These are, first, that the animal is under an anæsthetic and, second, that the afferent fibres in the nerve have all been cut. The

effect of an anæsthetic is almost certainly negligible, for it makes no difference to the rate or character of the respiration and the intact nerve discharge shows the same features in the decerebrate cat when the effect of the anæsthetic has worn off. The effect of cutting the afferent fibres will be dealt with later. Admitting, however, that the conditions are to this extent abnormal we may proceed to examine the single fibre discharge in more detail.

Duration and magnitude of electric responses. An analysis of the responses made with Lucas's instrument from the electrometer records shows the typical form of a wave of action potential in a single fibre, but our records do not give much information as to the precise duration of the response, since we have no exact information of the temperature of the nerve[1]. As in the case of sensory fibres the magnitude of the responses appears to depend entirely on the local condition of the nerve. They may vary from one record to another owing to the variable amount of fluid on the nerve and to the readjustment of the electrodes, but they show no sign of increasing in size when the respiration is more forcible. This may

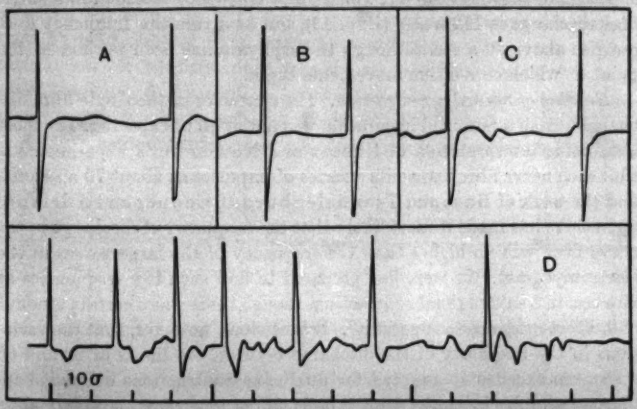

Fig. 9. Analysis of electrometer records showing constant size of action currents. *A*, *B* and *C*. Exp. 19. Nerve under constant conditions throughout. *A*. Normal breathing. *B*. A few minutes later. Air tubes clamped. Forcible respiratory movements. *C*. A few minutes later. Normal breathing. *D*. Exp. 13. Record at end of forcible inspiration with air tubes clamped.

[1] The nerve was irrigated with warm Ringer, but the air in the animal box was below 35° C., and the temperature of the nerve would fall off between successive irrigations.

be seen from Fig. 9 which gives the corrected curves from three records made in the space of a few minutes without touching the nerve. In *A* and *C* the animal is breathing normally and in *B* the air tubes have been clamped. Fig. 9 *D* is another example of the constant size of the responses. The record was taken at the end of a powerful inspiration with the air tubes clamped and it will be seen that although the frequency declines rapidly, there is no change in the size of the individual responses beyond the range of experimental error.

Frequency of impulses. Tables I and II show that the frequency in the discharge of a single fibre varies from about 20–30 a sec. during normal breathing to 50–80 a sec. in asphyxia. The impulses are spaced more or less evenly: the amount of departure from complete regularity varies from one preparation to another, but it is much the same in any one preparation whatever the frequency of the discharge. At the beginning and end of inspiration the intervals between the impulses are longer, but there is no distinct pause in the discharge except that between each period of inspiration. During this pause, which lasts about $\frac{1}{2}$ sec., there is no sign of nervous activity. The highest frequency recorded in a single fibre discharge is 112 a sec. (Exp. 13), but as a rule the frequency does not rise above 80 a sec. although the asphyxia has been pushed to the point at which convulsive movements begin.

Grading of muscular contraction. The character of the single fibre discharge during a powerful inspiration was not at all unexpected. The most reasonable interpretation of Gasser and Newcomer's experiments is that each nerve fibre transmits a series of impulses at about 70 a second, and the work of Bass and Trendelenburg(8), Cooper and Adrian(9) and others has made it most likely that the frequency of discharge in the nerve fibre was no higher than the frequency of the large waves in the electromyogram. We were less prepared to find such low frequencies as 20 a sec. in a submaximal contraction, though there was a certain amount of indirect evidence to suggest it[1]. It is obvious, however, that the variations in the frequency of the discharge between the limits of 20 and 80 a sec. are excellently adapted for producing contractions of graded intensity without bringing fresh muscle fibres into play. A mammalian muscle stimulated 20 times a second gives an incomplete tetanus, and the tension increases with the frequency of stimulation until the individual twitches have become completely fused. If all the muscle fibres are activated simultaneously, as they must be with stimulation of the motor

[1] Cf. the work of Wachholder(13) and of Richter(14) on the voluntary electromyogram.

nerve, the contraction will proceed in a series of jerks when the frequency of stimulation is below the fusion point; but if the different groups of muscle fibres are not synchronised the whole muscle will contract more or less smoothly. The irregular character of the discharge from the whole nerve in quiet respiration shows that there is, in fact, no well-marked synchronisation at the lower frequencies, and the diaphragm will therefore contract smoothly although the tension in each muscle fibre would show a series of peaks and depressions.

As the frequency needed for a smooth maximal contraction varies from one muscle to another, we have made a rough determination of the curve relating force of contraction to frequency of stimulation for the rabbit's diaphragm. In a rabbit anæsthetised with urethane and chloroform both carotids were tied and the cerebral circulation was occluded by a ligature tied tightly round the neck below the transverse process of the atlas. The respiration was maintained artificially and discontinued for short periods when the phrenic was stimulated. The 4th cervical root of the nerve was used instead of the 3rd as it produces a more powerful contraction. Stimulation was by a series of break shocks from a coreless induction coil connected with a rotating interrupter, the speed of which could be read directly by a magnetic speedometer. The force of contraction was recorded by connecting the air tube from the trachea to a water manometer, the reading being taken after 2–3 sec. stimulation when the pressure had reached a steady value.

A curve made from a typical set of readings is shown in Fig. 10. The

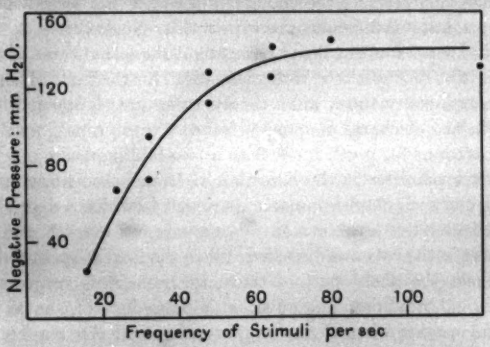

Fig. 10. Exp. 20. Negative pressure in thorax produced by stimulating 4th cervical root of phrenic at different frequencies. Frequencies varied in random order.

method of recording may give an exaggerated idea of the actual change in average muscular tension at the different frequencies, but it shows well enough that the suction caused by the diaphragm is approximately doubled when the stimulation frequency rises from 25 to 60 a sec. and that there is little further change when the rate exceeds 60 a sec. Thus the frequencies which we find in the single nerve fibre cover just the range which will give the maximum variation in the force of contraction.

It has been generally assumed that in a submaximal contraction of reflex origin only a portion of the muscle is in action, more and more motor neurones taking part as the contraction increases in force. It is, therefore, of interest to enquire how far this method of gradation is made use of in the rabbit's diaphragm to supplement that provided by the variation in the frequency of the discharge. The answer must be that as far as concerns that part of the muscle which is supplied by the 3rd cervical root of the phrenic there is no marked increase in the number of neurones in action when the contraction becomes more forcible, the gradation being due mainly to the increased number of impulses which reach the muscle from each nerve fibre. If many of the nerve fibres are only brought into play with forcible breathing we should expect to find some preparations in which the few remaining nerve fibres transmit impulses only when the air supply is cut off. Actually in all the experiments where we have obtained single fibre discharges these have occurred in quiet breathing as well as in asphyxia. In some of the experiments where a greater number of fibres were intact the increased discharge during asphyxia seems large enough to indicate the accession of fresh nerve fibres, but it is difficult to be sure of this. No doubt an examination of the other nerve roots of the phrenic might show that some of the fibres are only active during a powerful contraction, for the force of contraction must increase many times when the air tubes are clamped, whereas an increase in the discharge frequency from 20 to 60 a sec. would not be likely to account for much more than a two-fold increase.

Evidence pointing to the accession of fresh nerve fibres in forcible breathing has been obtained in an experiment in which a decerebrate cat was used instead of a rabbit. In this preparation the 4th cervical root (the highest in the cat) was dissected. When the final stage in the division was reached, the distal part of the nerve remained completely quiet during normal breathing, but a distinct impulse discharge could be heard in the loud speaker when the air supply was cut off. After a few minutes an impulse discharge appeared during normal breathing as well, indicating that some fibres had been temporarily thrown out of action by pressure

during the dissection and had now recovered their power of conduction. For this reason the experiment is scarcely conclusive, for the appearance of impulses only in forcible breathing might have been due to summation occurring in a partially damaged fibre.

In the experiments on the rabbit we have looked for evidence of the accession of fresh fibres as the contraction develops by comparing the duration of the single fibre discharge with that of the whole nerve. If more and more fibres come into play during the course of inspiration, the discharge of the whole nerve would usually outlast that of the single fibre, for it is unlikely that the fibre we isolate would always turn out to be one which comes into action at the beginning of inspiration. In general, however, we have found no clear evidence of the single fibre discharge being much shorter than that of the whole nerve. The average duration of both is about $\frac{1}{2}$ sec. in normal breathing, and there is no indication of a constant difference in length in any one experiment. It must be admitted that a difference as large as 20 p.c. might pass unnoticed, for the duration of the discharge varies somewhat in the course of the experiment.

Synchronous activity of motor neurones. When the impulses in each fibre recur at a frequency as low as 25 a sec. the contraction of the diaphragm would proceed in a series of jerks if there were an exact synchronisation of the discharge in the different nerve fibres. Records such as those in Figs. 4 and 8 show that there is some tendency for the impulses in different fibres to occur at the same moment but this is not great enough to prevent an almost constant succession of impulses from reaching the muscle. On the other hand, when the frequency in each fibre rises to 60 a sec. a smooth contraction will result whether the different neurones are discharging independently or by volleys, and it is interesting to find that the greatest degree of synchronisation does in fact occur in these rapid discharges where it will have the least effect on the smoothness of the contraction.

The synchronisation at high frequencies can be seen from a comparison of the single fibre discharge with that of the whole nerve. When the single fibre gives a frequency of 50–80 a sec. the discharge of the entire nerve becomes of the type in which there is a more or less regular series of large waves at 50–80 a sec. An example of this is shown in Fig. 11, *A* and *B*, and there can be little doubt that the discharge of the whole nerve is built up of more or less synchronous volleys.

The degree of regularity in the size and frequency of the waves in the whole nerve discharge, and therefore the degree of synchronisation, varies

from one preparation to another. Fig. 11 *C* is an example of an extremely regular discharge from an entire nerve (Exp. 7). Below it (*D*) is the dis-

Fig. 11. Comparison of discharge of intact nerve with that of a few fibres. Air tubes clamped to produce regular rhythm. *A* and *B*. Exp. 13. *A*. Electrodes close together above the dissected area. *B*. Electrodes below the dissected area. The record shows individual impulses, most of which appear to form a single series. *C* and *D*. Exp. 7. *C*. Before dissection. Electrometer one-sixth maximum sensitivity. *D*. After dissection. Several fibres in action. Maximum sensitivity. Time marker gives ·125 sec. intervals.

charge of two or three fibres which were left intact, the sensitivity of the recording system being six times as great as in *C*. Even here the synchronisation is not absolute, for it is occasionally possible to detect more than one impulse in the waves of the lower record. There is no doubt that the chief condition for the synchronous activity of the neurones is that the discharge should be near its maximum intensity, but we have not been able to make out what other conditions may favour or hinder its appearance.

Influence of afferent fibres. A factor which might be likely to affect both the synchronisation and the frequency of the discharge in our records is the absence of the proprioceptor impulses which normally pass up the nerve. Dusser de Barenne(10) and others have shown that the frequency of the large waves in the electromyogram may be reduced by

30 p.c. or more when the muscle is deafferented and the regularity may become greater when the frequency is reduced by painting the dorsal part of the cord with novocaine. It was possible therefore that the discharges we have obtained are of lower frequency and greater regularity than they would have been had the afferent fibres been undivided.

Though we cannot deny the importance of the afferent fibres we do not think that the general character of the discharges has been much affected by their destruction. The greater part of the proprioceptor supply from the diaphragm has been left intact, for the 3rd cervical root is only a small part of the phrenic, though it must be admitted that the afferent fibres which have been divided are those which are most intimately connected with the motor fibres we have studied. But we can form some estimate of the extent to which the discharge is affected by comparing it with that occurring in the nerve before it has been severed from its connection with the diaphragm. The records obtained by leading off from a nerve trunk which preserves both central and distal connections are complicated by the fact that they will contain afferent as well as efferent impulses and that they may be affected by changes of potential occurring in other parts of the body. The chief disturbance is that due to the heart, but fortunately the electrocardiogram is easily recognisable and it can be reduced in magnitude by adjusting the position of the electrodes on the nerve. We have recorded the discharge of the nerve in continuity in three experiments and compared it with that from the same nerve after cutting distally or from the cut nerve on the opposite side of the body. In no case was there any marked change in the frequency or regularity of the record. With quiet breathing the discharge has the usual irregular character with occasional large waves, and with asphyxia there was the usual increase in the number of the large waves, the dominant frequency reaching the typical value of 70–80 a sec. In none of these experiments with the nerve cut or uncut was the asphyxial discharge as regular as that shown in Fig. 11 *C*, and it is difficult to make exact comparisons in records which fall short of complete regularity; but the well-marked change in the character of the discharge as between quiet breathing and asphyxia is fairly clear evidence that the change from asynchronous to synchronous activity takes place whether the afferent fibres are intact or not.

It may be that the frequency in each nerve fibre is slightly higher when the afferent fibres are intact, but our records give no indication of this for the large waves occur at much the same rates with the nerve cut or uncut.

In this connection the experiments of Gasser and Newcomer may be recalled, for they too recorded the discharge in the nerve in continuity and found it to have the same dominant frequency as in a nerve cut distally. Moreover, they made simultaneous records from the right phrenic cut distally and the left half of the diaphragm with intact nerve supply and were able to detect corresponding waves in the two records (11). Here therefore the discharge of the right phrenic with its proprioceptor fibres cut through is synchronised with that of the left phrenic which is still connected with the diaphragm.

Discussion.

It is unlikely that the innervation of the diaphragm differs much from that of an ordinary skeletal muscle, and we may expect to find that the discharges of other motor neurones will have the same general character as those of the phrenic. But there is no reason why the same method of dissection should not be applied to the nerves supplying the skeletal muscles, and until we have done this it would be premature to discuss our results as though they had been found in all kinds of reflex discharge. To mention only one possibility, it may be that a group of motor neurones which are activated by a centre in the brain stem will give a different type of discharge from those activated in a short spinal reflex. At the same time it will be surprising if such a finding as the gradation of contraction by the change in frequency of the discharge does not turn out to be generally applicable, for it removes certain difficulties which arise from the view that gradation is mainly due to a change in the number of fibres in action. This is the most interesting, because the least expected, of our results. For the present, however, we may treat the phrenic discharge as a good example of the general principles which seem to govern the activity of the neurone, without implying that other motor discharges may not differ considerably in detail.

Previous work has shown that in the sensory nerve fibres the impulses produced by stimulation of the end organ are of fixed size and that they recur at more or less regular intervals with a frequency which is determined by the intensity of the stimulus, but is never high enough to prevent the fibre from recovering after the passage of each impulse. The present investigation shows that the motor fibres of the phrenic transmit discharges which are almost an exact counterpart of those in the sensory fibres. The impulses (or rather their action potentials) obey the same all-or-nothing principle; their frequency varies with the intensity of the central excitation, and varies over much the same range as in the sensory

fibre. The upper limit of the motor discharge in the phrenic is somewhat lower than that from a sensory end organ (which may reach as much as 150 a sec.), but an increase to higher rates would be of no obvious value in the motor fibre, for it would cause no further increase in the force of contraction. If we compare the sensory discharge in a small nerve with the motor discharge in a few fibres of the phrenic the chief difference is that each sensory fibre acts independently so that the impulses in the different fibres are distributed at random, whereas in the motor fibres there is often a considerable degree of synchronisation so that the impulses occur in groups (cf. Fig. 8). It may turn out that a synchronous discharge of this kind is only met with when we have a spinal centre acting under the control of the brain stem or cerebrum. If the discharge from the higher centre is conveyed by a few nerve fibres to a large number of motor neurones, a group of adjacent neurones may discharge in unison because the whole group is innervated by a single fibre from above. But the fact that the entire phrenic (in Gasser and Newcomer's experiments) gives a discharge in volleys shows that somewhere in the central nervous system (either in the respiratory centre or in the phrenic nuclei in the cord) the neurones must be linked together to work in unison. It was to obtain some evidence on this point that our research was originally planned. In a recent paper on the optic nerve Adrian and Rachel Matthews(11) described a rhythmic type of discharge occurring when the entire retina is evenly illuminated, and concluded that it was due to a synchronous activity of the different ganglion cells. It was suggested that the development of a synchronous beat might be a natural reaction in any large group of neurones connected with one another and uniformly excited, and the existence of a dominant rhythm in the electromyogram of a powerful contraction was mentioned in support of this. The present results bring the motor discharge in the phrenic into line with the discharge of the optic nerve in so far as they show the synchronous working of the motor neurones in forcible inspiration.

In both cases a feeble or restricted excitation gives an asynchronous discharge, but the explanation advanced for the retina (lack of uniformity in the excitation of the different ganglion cells) will scarcely apply to the phrenic where there is no evidence that a feeble excitation is not uniformly distributed. If it were not uniform we should expect to find much greater variation in the single fibre frequencies in quiet breathing. For this reason the precise mechanism which determines the synchronous discharge must be left until more evidence is available.

We need not discuss the whole question of the voluntary and reflex

electromyogram and the relation it bears to the nerve discharge until we have examined other nerves than the phrenic. In this particular case Gasser and Newcomer's experiments had already shown that the electromyogram is a fairly accurate copy of the electroneurogram, and that the frequency of the large waves gives the frequency of the volleys which are discharged by the motor nerve cells. In discussing the reflex electromyogram in the frog Cooper and Adrian concluded that the small waves in the record were produced by the activity of a few units acting out of phase with the majority, as Piper had originally suggested. This conception was embodied in a diagram (Fig. 7 of their paper(12)). For the action current record of the phrenic this diagram could be applied without any modification, and indeed it bears a striking resemblance to the actual records in Fig. 4 of the present paper.

Summary.

Records have been made of the electric responses in single motor fibres of the phrenic nerve of the rabbit during the normal respiratory discharge. The highest root of the phrenic was almost completely divided with fine needles so that only three or four fibres remained intact and the electric responses in the nerve distal to the division were examined with the capillary electrometer and valve amplifier. In five experiments the dissection of the nerve was carried to a stage at which a single series of impulses appeared in the records. From these and others where more fibres were in action we draw the following conclusions:

(1) With normal breathing the impulses in each nerve fibre recur at a frequency of about 20–30 a sec. throughout the period of inspiration.

(2) When forcible inspirations are produced by clamping the air tubes to the trachea the frequency of the impulses rises to 50–80 a second, the highest recorded frequency being 112.

(3) The size of the action currents remains unaltered when the force of inspiration is increased or reduced, the only change being in the frequency of the discharge.

(4) With low frequencies of discharge, as in normal breathing, the different nerve fibres do not work in unison, though there is some tendency towards a grouping of the discharge. With the high frequencies (in forcible breathing) there is a much greater synchronisation, and the discharge consists of successive volleys from the majority of the nerve cells.

(5) The grading of the contraction in the diaphragm appears to depend mainly on the changes in the frequency of the impulse discharge in each nerve fibre. At low frequencies each group of muscle fibres will

give an incomplete tetanus, but as the groups are not all working in unison, the whole muscle will contract smoothly. When the phrenic is stimulated artificially by a series of shocks an increase in frequency from 25 to 60 a sec. doubles the suction produced by the diaphragm.

(6) It is possible that fresh nerve fibres are brought into play when the force of inspiration is increased, but this method of gradation does not seem to have much importance in comparison with that due to the change in discharge frequency.

(7) The electric response of the entire nerve consists of a succession of large waves which vary in size and regularity according to the force of the respiratory movement, and are due to a more or less synchronous discharge in a number of fibres; and of small waves, more prominent in quiet breathing and due to fibres working out of phase with the rest but at much the same frequency.

(8) Though some of the afferent fibres from the diaphragm were cut through in our experiments, it does not appear that the general character of the impulse discharge has been much affected by their loss, for the discharge of the whole nerve is of the same type whether the distal connections are intact or not.

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THE EFFECT OF ALCOHOL ON THE ABSORPTION OF GLUCOSE FROM THE ALIMENTARY TRACT.
Part II.

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In Part I of this communication (1) it was pointed out that the only satisfactory way of studying absorption from the alimentary tract of such substances as glucose would be to recognise the presence of the absorbed substance in the blood stream and not rely simply upon its disappearance from the gut. In the case of sugar absorption this presents difficulties when working with anæsthetised or decerebrate animals because of the attendant variations of blood sugar evoked by the technique employed. There remain two alternatives, either to work with normal unanæsthetised animals or to use some hypnotic which does not cause variations in the blood sugar level. The first of these methods is naturally ideal, and some preliminary experiments on normal rabbits showed that giving a constant amount of glucose caused the rate of absorption, as estimated by the blood sugar level, to be greater when alcohol was simultaneously supplied. This method was however rejected since different individual animals showed considerable variations at different times. Recourse was had therefore to animals under amytal (ethylisoamylbarbituric acid) for the production of anæsthesia, since this substance is stated by Page (2) to maintain deep anæsthesia without producing hyperglycæmia. Moreover, it gives, according to Deuel, Chambers and Melhorat (3), after an initial fall a constant blood sugar level, slightly lower than the normal, over periods as long as six to eight hours.

In the series of experiments about to be described amytal was employed as the anæsthetic throughout.

Method.

Cats were employed after 18 hours' deprivation of food, and without any preliminary anæsthetic were given an intraperitoneal injection of a 10 p.c. solution of amytal, made by dissolving 1 grm. of amytal in 8·85 c.c. half normal NaOH with gentle heating and adding water to 10 c.c. The

dose for the production of anæsthesia in a cat was 0·8 c.c. of this solution per kilo of body weight. Almost invariably the animals went under in ten minutes. Great care was taken during the experiments to keep the animals warm by means of rubber hot water bottles and flannels kept hot by electrical means. (It may be mentioned that amytal is said to lower the body temperature.) After tracheotomy the carotid artery on one side was exposed but not ligatured. A tube was tied into the œsophagus at the neck. A canula was placed in the femoral vein by which injections could be made. Directly these operations were completed a sample of blood was taken by a syringe from the carotid artery and the hole so made closed by a clip without occlusion of the vessel, thus the cerebral circulation was in no way interfered with. After the lapse of half an hour another blood sample was taken and the glucose solution introduced. The quantity of glucose given was comparable to that of the glucose tolerance test, that is, 2 grm. per kilo body weight in a concentration of 20 p.c. This solution was administered by means of the tube tied into the œsophagus and the remains were washed in with half the volume of water or, in experiments with alcohol, half the volume of 30 p.c. alcohol. Thus a final concentration in the gut was reached of about 13 p.c. glucose. Blood samples of 0·8 c.c. were taken at definite intervals in the way described above, and were followed by the immediate injection of an equal amount of saline to make up the blood volume.

The sugar was estimated by the method of Hagedorn and Jensen, employing 0·2 c.c. of blood and performing each estimation in triplicate.

The experiments fall into three series.

Series I.

Realising that there would probably be wide differences in the amount of alimentary hyperglycæmia caused by the administration of glucose in different animals, it was anticipated that a rather large number of experiments would have to be done, in some cases with glucose alone, in others with glucose and alcohol, in order to obtain a reliable average for any conclusion to be drawn with precision. The first set of experiments were carried out as follows. Glucose was given and the blood sampled at regular intervals until the sugar level was either constant or falling slightly, then alcohol was introduced in the quantity and concentration described above and the blood sugar again determined at intervals of half an hour. The expectation was that when the blood sugar was at its maximum height alcohol would, if it aided absorption, cause a further rise. This

anticipated rise was observed to take place as shown in the table of observations and graphs annexed.

Experiment	Initial blood sugar (mg. per 100 c.c.)	Maximum blood sugar with glucose alone	Maximum after alcohol
III	136	187	326
V	138	278	318
VI	120	210	252
VII	103	193	215
XI	129	197	242
XII	163	209	255
Average	132	212	268

Graphs of the course of changes in III, V and XI are presented in Fig. 1.

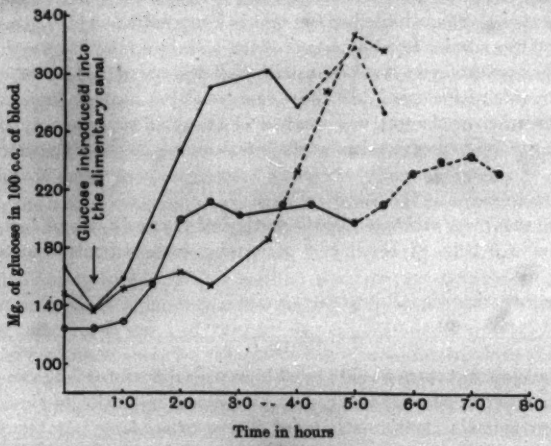

Fig. 1.

After introduction of glucose into alimentary canal ———
After introduction of alcohol into alimentary canal - - - - -

In considering these results there is first the obvious criticism that the effect of the alcohol when absorbed might have been to turn out glucose from the glycogen of the liver and muscles, and that the increase did not represent augmented absorption. If this were so, then alcohol introduced directly into the blood stream should cause a similar, or even a greater,

increase. In two cases when alcohol was introduced directly into the blood stream (*via* the carotids) a fall and not a rise was obtained. But to show definitely that the alcohol was not acting by glycogen mobilisation several experiments were made where alcohol alone was passed into the œsophagus and the effect on the blood sugar level noted. The alcohol would be very rapidly absorbed in a fasting animal (see E. Mellanby (4)). Nevertheless no marked rise resulted, as shown in the next series of observations.

Series II.

These experiments were designed to show the effect of amytal alone, and further, the effect of alcohol alone on the blood sugar level. Graphs of this series are presented in Fig. 2.

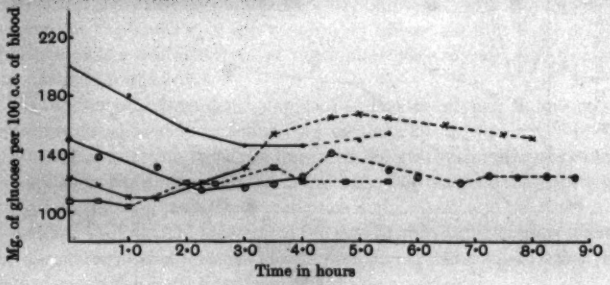

Fig. 2.

After injection of amytal ———
After introduction of alcohol into alimentary canal - - - - -

From these graphs it can be seen that the invariable effect of amytal is to produce an initial fall in blood sugar and then maintain a constant level in cats as in dogs. (See Deuel, Chambers and Melhorat (3).) The giving of alcohol alone causes but a very slight rise in the sugar level.

Series III.

Having obtained evidence that alcohol aids the absorption of glucose and that it increases postprandial hyperglycæmia, another series of experiments was performed to confirm the point definitely. In these a complete record was made of the rise of blood sugar due to giving glucose as described and, wherever possible, the return of the blood sugar to the original value. In short, these experiments were similar to the glucose tolerance tests in man. A fairly large number of experiments on these lines proved

to be necessary before any generalised statement could be made and before a standard could be fixed with which to compare results obtained in similar experiments where, in addition to sugar, alcohol was also given.

The results are best seen in the following graphs in Fig. 3.

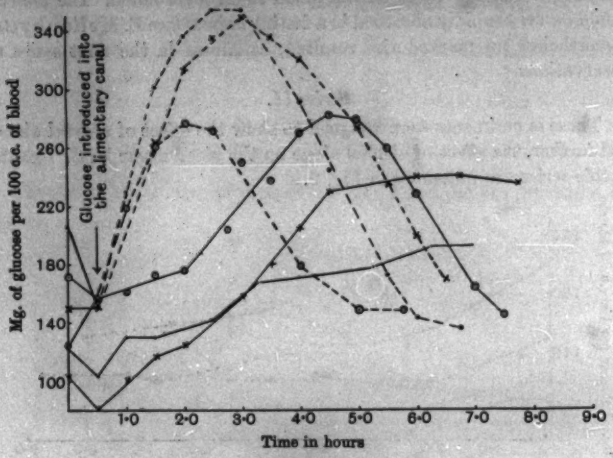

Fig. 3.

After introduction of glucose into alimentary canal ———
After introduction of glucose and alcohol into alimentary canal - - - - -

To facilitate the interpretation of these results several points require emphasis.

(1) The maximum sugar level occurs on an average (taken from a larger number of experiments than charted) four hours after the giving of the sugar, *i.e.* the absorption is slow.

(2) The average maximum figure for all experiments with glucose in the absence of alcohol is 232 mg. per 100 c.c.

(3) The fall in the blood sugar level is very slow, so slow that in most cases it was not possible in the experiments to follow the fall.

No doubt the reason of this maintenance of the hyperglycæmia is due to the lowered metabolic rate which is a characteristic effect of amytal, and also possibly to a decrease of the glycogenic function of the liver under this drug. That amytal does diminish the activity of the glycogenic function of the liver has been shown by Hindes, Boyd and Leese(5).

The experiments to be compared with these were carried out under exactly the same conditions. Proportionally the same amount of glucose was given and also a volume of 30 p.c. alcohol equal to half the volume of the glucose solution, resulting in a concentration of 10 p.c. alcohol. Again the results are presented graphically (see Fig. 3).

Emphasising the same points as before, we have: (1) the maximum sugar level occurs on an average two hours after giving sugar together with alcohol, (2) the average maximum sugar level is 311 mg. per 100 c.c., (3) the fall of the sugar level is more rapid. The explanation of the more rapid fall is not altogether clear. It might be that alcohol stimulated the metabolism and so led to a utilisation of the sugar or, that glycogen became formed. These possibilities could only be determined by observations of the metabolic rate and respiratory quotients. But leaving for the present the explanation of this more rapid fall we see that there is clear evidence of the augmentation of sugar absorption by the simultaneous administration of alcohol.

Whatever criticisms can be applied to results relating to absorption experiments obtained by estimating the rate of disappearance of the substance in question from the lumen of the gut, such criticisms cannot be applied to the experiments recorded here. The disappearance from the gut is definitely related to the appearance in the blood, and the conclusion is justified that alcohol accelerates the rate of absorption of glucose from the alimentary canal into the blood. Similar experiments are being performed along the same lines in normal human subjects.

Summary.

1. If glucose is introduced into the alimentary canal of an animal under amytal anæsthesia the blood sugar rises and reaches a maximum in about four hours after which it very slowly falls.

2. If alcohol is introduced with the glucose the maximum sugar level is reached in about two hours.

3. The average maximum level without alcohol is 232 mg. per 100 c.c. of blood; with alcohol it is 311 mg. per 100 c.c. of blood.

4. The blood sugar falls much more slowly to the normal value when alcohol is not given.

5. The influence of alcohol alone on the blood sugar level is shown to cause a slight rise.

6. Introduction of alcohol after the maximum level of alimentary hyperglycæmia with glucose alone has been reached causes a further rise in the sugar level.

7. From these observations it is concluded that the effect of alcohol is not greatly to mobilise liver glycogen but to influence directly the rate of absorption of glucose from the gut.

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THE INCIDENCE OF CENTRAL INHIBITION ON RESTRICTED FIELDS OF MOTOR-UNITS.

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INTRODUCTION.

RECENT work by Sherrington and his collaborators (8, 6, 7; also 5, 9) has made it clear that when a muscle contracts reflexly in response to electrical stimulation of an afferent nerve, a fraction only of the total number of its contractile elements is as a rule, even with maximal stimuli, thrown into activity. Different afferent nerves can excite larger or smaller fractions of the given test muscle. By concurrent stimulation of two such nerves, it has been shown that some contractile elements are activated by only one of them while others are common to them both. The extent of this overlap between the two fields of "motor-units" (15) with which the afferent nerves are in functional connection, varies both with the condition of the spinal cord and with the intensity of the stimuli employed. With strong stimuli, the field excited by each afferent nerve acting alone is larger, and the overlap between two fields is greater (relatively as well as absolutely), than with weak stimuli (7). Moreover, the field proper to any afferent nerve is scattered in many separate and widely distant muscles, all of which, by contracting together, constitute a single executive unit. The use of the word field in this connection must not, of course, be taken to imply anatomical contiguity of the motor neurones or contractile elements concerned. It is used in no geographical sense and merely refers to the unity of action and similarity of threshold, with respect to the given afferent nerve, of a number of synergic motor-units whose neurones may be scattered through many segments of the cord. Both the extent and the distribution of the field vary with different afferent nerves and are characteristic of those nerves. The term "Fractionation" has been applied (6) to this functional splitting up of a "half-centre" (1, 2) or of a muscle between the various afferent nerves which are excitatory to it.

Though its nature and mechanism are less perfectly understood, inhibition is now universally recognised to be as characteristic of most

reflex actions as is excitation. It therefore seemed of interest to determine (*a*) whether inhibitory fractionation of motor-units occurs when contracting muscles are reflexly inhibited by electrical stimulation of appropriate afferent nerves, or (*b*) whether the inhibition is distributed among all the active units. In either case, each individual active unit will become quiescent under the inhibitory influence so soon as this attains a strength equivalent to that of the concurrent excitatory influence[1]. If the second alternative be true, the extent to which the inhibition manifests itself by partial or complete muscular relaxation will depend merely on the number of active units in which the interplay of these opposing influences results in an excitatory deficit. But if the first be the correct solution, it depends also on the amount of overlap between the excitatory and inhibitory fields, and some of the active units may not be subjected to any inhibitory influence at all.

That the "inhibitory field" may include almost the whole of one spinal half-centre when a strong stimulus is applied to an afferent limb-nerve, is indicated by familiar observations on compound reflexes. Thus when a crossed extension reflex is elicited in a decerebrate cat by faradisation of a twig of the contralateral internal saphenous nerve, both the resulting contraction and the initial tonus of quadriceps extensor may be completely inhibited by stimulation of a branch of the ipsilateral hamstring nerve (13). In this particular example, the complete overlap of the excitatory by the inhibitory field is especially striking in view of the different segmental origins of the two afferent nerves employed.

The paucity of published observations having any bearing on the question under consideration, other than a number of the type described in the last paragraph, is due to the difficulty of determining the incidence of inhibition on inactive motor-units. We can only get an idea of the extent and intensity of central inhibition by observing its effects on a coincident excitation. This gives us some measure of what Graham Brown (2) has appropriately termed "effective inhibition" (*i.e.* the portion of inhibition which falls upon active neurones) but not of "total inhibition" (*i.e.* the mass of inhibition which falls upon any neurones, whether active or inactive, in a half-centre). It is the distribution of the latter

[1] That "algebraical summation" of excitation and inhibition occurs in individual motor-units is indicated by experiments in which a constant inhibitory stimulus is found to produce marked relaxation when pitted against submaximal excitatory stimuli, but to cause no relaxation of more powerful reflex contractions. Such observations have been reported by Sherrington (18) (Fig. 6) and by Graham Brown (3) using isotonic recording, and are confirmed with an isometric myograph by some of the experiments recorded in this paper.

which is the subject of this paper and which can only be inferred indirectly from that of the former. The following example, however, may be taken to provide some evidence in favour of inhibitory fractionation.

Liddell and Sherrington(11, p. 227) investigated the ipsilateral knee-extensor contraction reflex which is sometimes elicited by stimulation of such afferent nerves as peroneal or popliteal. They showed that, as judged by its effect on the test muscle, the afferent nerve in these cases must be regarded as a mixed trunk, containing a relatively small number of excitatory among a large number of inhibitory nerve fibres. The preponderating effect of the latter is manifested when the nerve is stimulated concurrently with a contralateral limb nerve. Under these conditions, the contraction of the knee-extensor in the crossed extension reflex is markedly inhibited, though the relaxation does not, during the concurrent stimulation, extend fully to the base-line given by the muscle when at rest. But if the ipsilateral nerve alone is stimulated, the effect of the excitatory fibres is manifested and some contraction is evoked. The height of this contraction corresponds exactly with the level above the base-line at which the inhibitory fall is checked during the compound stimulation. "The inhibitory effect, potent as it is against the excitatory effects of other nerves and of the stretch-reflex, remains impotent against the excitatory effects of some of the fibres of its own nerve." Though there are other possibilities, it is plausible to suppose that the two sets of fibres make connection with different fields of motor-units.

The argument.

The line of reasoning on which our experiments are based will be most easily followed by considering the following statement made by Sherrington(15). "It sometimes happens that the same strength of stimulus of the inhibitory afferent evokes more relaxation when pitted against a larger reflex contraction than against a smaller one." This observation is capable of two interpretations.

On the one hand, both the excitation and the inhibition may be acting on restricted fields of motor-units, *i.e.* fractionation is occurring with respect to both afferents. Then with strong excitatory stimulation the excitatory field will overlap the inhibitory field to a greater extent than does the smaller excitatory field with weak stimulation. On this hypothesis, if the stimuli applied to both nerves are sufficiently weak, it may happen that no overlap occurs between their respective fields. The inhibitory afferent will then be powerless to cause any relaxation of a contraction evoked from the excitatory afferent. When the strength of

the excitatory stimulus is now increased while that of the inhibitory is kept constant, the excitatory field will increase in size and will begin to overlap the inhibitory. At this point inhibition will manifest itself by small muscular relaxation. Further increase in size (within limits) of the excitatory field will be accompanied by greater opportunity for the inhibitory effect to show itself. The ratio of the relaxation to the total size of the excitatory field will increase as the strength of the excitatory stimulation is increased.

On the other hand, Sherrington's result would also be obtained if the inhibition were evenly distributed throughout the half-centre of which the excitatory field is a part. For suppose the motor-units constituting the excitatory field to be removed from their normal anatomical distribution and to be rearranged diagrammatically in a circle, the area of which is proportional to the number of motor-units which it contains. The most intensely excited units are at the centre and the least intensely excited are at the circumference. A uniformly distributed weak inhibitory influence will be strong enough to abolish the activity of the latter but not of the former, and the resulting relaxation will be proportional to the area of the ring so inhibited. Any increase in the size of the original circle, such as would be produced by increasing within limits the strength of the excitatory stimulation, will also increase the area of the ring, whether the width of the latter (*a*) be constant, or (*b*) be proportional to the radius of the circle. But a simple calculation applied to this diagram shows that the ratio of the area of the ring to the area of the original circle (*a*) diminishes or (*b*) remains constant as the radius of the circle increases. When the circle reaches its limiting size, *i.e.* when the stimulation of the excitatory nerve engages all the units with which that afferent can make connection, further increase in strength of the excitatory stimulus can merely make the units near the circumference of the circle more resistant to inhibition. The area of the inhibitable ring is in fact reduced, so that in this case again the ratio of the area of the ring to the area of the original circle diminishes as the strength of the excitatory stimulus increases.

If, therefore, experiment should show that, when an inhibitory stimulus of constant strength is thrown on backgrounds of successively graded excitations, the relaxations produced, expressed as percentages of the background contractions, increase with increase in the size of the excitatory field, the only possible conclusion would be that the inhibition is falling on a fraction only of the motor-units of the half-centre, and that this fractional field is being overlapped more and more by the successive excitatory fields. The result will not be obtained if either field completely

overlaps the other with the weakest strength of excitation employed. It is therefore essential to use weak inhibitory stimulation and to begin with excitatory stimuli only just above the reflex threshold. Once the excitatory field is large enough to include all the units subject to inhibition, further increase in its size will be accompanied by decreasing percentages of inhibition.

It should be emphasised that this argument is based on a diagram which does not profess to be an actual picture of the conditions within the central nervous system. Such a diagram must be used with caution and is only of value in the solution of a few particular problems. But even if some of the assumptions made above are unjustifiable, there remains one unimpeachable criterion by which the occurrence of inhibitory fractionation may be tested. An experiment in which a constant inhibitory stimulus has absolutely no effect on a small reflex contraction while causing obvious partial relaxation of a larger contraction, can only be explained by saying that the smaller excitatory field is not subjected to the inhibitory influence which manifests itself in the larger excitatory field. In other words, the inhibition is confined to a restricted field in the half-centre, and this field is independent of the smaller of the two excitatory fields.

Technique.

All our observations have been made on decerebrate cats. Under deep anæsthesia one hind limb was completely immobilised and its skin denervated by appropriate nerve and muscle section. The same procedure was followed on the other side except that one flexor muscle, either *semitendinosus* (knee flexor) or *tibialis anticus* (ankle flexor), was left with nerve supply intact. In each limb a number of nerves were ligated prior to cutting them and their central ends prepared for electrical stimulation. The tendon of the test muscle was severed close to its insertion and prepared for the myograph. The fleshy part of the muscle was dissected clear of surrounding structures so far as was compatible with the integrity of its nerve and blood supply. The animal was then decerebrated at a level just in front of the posterior colliculi by the trephine method and left for two hours for the effects of the anæsthetic to pass off.

In the myograph room the hind limb containing the test muscle was securely fixed to the table top by means of steel drills inserted into the ends of the femur (or, when *tibialis anticus* was used, into the ends of the tibia). The muscle was arranged to have a vertical pull with its tendon connected to the myograph lever by a light steel hook. The myograph was an isometric one of the Sherrington high-frequency torsion-wire type

with optical recording system, as described in previous communications from this laboratory (14, 10). In each experiment the resting muscle was given a slight initial stretch (about 15 grm. tension) and its length was then kept constant throughout the series of observations.

Afferent nerves of the two hind limbs were stimulated faradically by about 50 double shocks per second delivered from coreless Berne induction coils fed by two volts in the primary circuits. The shadows of short-circuiting keys in the secondary circuits were thrown on the slit of the falling-plate camera by the same source of light as was that of the myograph pointer. In most of the experiments the electrodes applied to the nerves were of a new fluid pattern devised by one of us (J.C.E.) and described in detail in the appendix to this paper. They were put in position immediately after decerebration and generally required no further attention before the end of the experiment seven or more hours later. Their use enabled us to take long series of comparable observations in which the same strength of stimulus applied to the same nerve could be relied on to evoke approximately the same size of reflex response. With ordinary hand electrodes and a nerve suspended in air, the recording of regular series of very small reflex contractions is almost impossible. The excitability of the nerve varies with the position of the electrodes, and some changes due to drying and cooling are inevitable.

Results and discussion.

The test flexor muscle was made to contract reflexly by stimulation of an afferent nerve of the ipsilateral limb. After about 0·4 sec., when a steady plateau had been recorded, a concurrent weak stimulus was applied to an afferent nerve of the contralateral limb and the compound stimulation maintained for about 0·4 sec., by which time a second plateau was reached. The inhibitory stimulus was then withdrawn and the ipsilateral nerve alone left in action for a further period of 0·4 sec. In this way series of records were obtained of the inhibitory effect produced by a weak inhibitory stimulus of constant intensity, acting against a background contraction the tension of which varied from zero to any desired number of grams short of the maximal reflex tension obtainable from the given excitatory afferent. The backgrounds actually used were always far smaller than the maximal reflex response obtainable, because, as explained above, we were only interested in small contractions. Graham Brown (2, 3) has published similar series showing constant inhibition acting against graded excitation. His observations were however made for another purpose and do not include a sufficient number of small excitations to

enable a comparison to be made with ours. He further employed isotonic recording, which, owing to the changing lengths of the individual muscle fibres at successive stages of the experiment, makes a comparison of his statistical results with ours difficult.

As control observations, we recorded in each experiment the effect both of the ipsilateral and of the contralateral stimulation acting alone. In a few cases the test muscle contracted in response to the crossed stimulus (4); all observations on preparations showing this contralateral flexion are discarded. "Mixed" ipsilateral afferents could not be so easily detected, but the possibility of the occurrence in the excitatory afferent of a few fibres with an inhibitory effect does not invalidate our conclusions. The internal saphenous nerve was as a rule found to be unsatisfactory as an excitatory afferent owing to the unsustained character of the reflex response.

Tables I, II and III give the figures obtained in three preparations:

TABLE I.

Tibialis anticus with graded stimulation of musculo-cutaneous branch of ipsilateral peroneal nerve. Maximal reflex tension about 1500 grm. (6).
Constant stimulation of contralateral popliteal nerve from second coil at coil distance 16 cm.

Sequence of observations	Coil distance for excitatory afferent, cm.	*A*. Tension developed from excitation alone in grm.	*B*. Fall in tension during inhibitory stimulation in grm.	*B* expressed as percentage of *A*
5	25	35	0	0
7	25	40	0	0
3	24	70	10	14
2	22	145	45	31
6	20	150	60	40
1	20	195	110	54
4	18	200	80	40
8	17	330	115	35

TABLE II.

Semitendinosus with graded stimulation of ipsilateral popliteal nerve (hand electrodes) Maximal reflex tension about 1500–2600 grm. (6).
Constant stimulation of contralateral internal saphenous nerve at coil distance 11 cm.

Sequence of observations	Coil distance for excitatory afferent, cm.	*A*. Tension developed from excitation alone in grm.	*B*. Fall in tension during inhibitory stimulation in grm.	*B* expressed as percentage of *A*
3	23	230	15	6·5
4	20	590	80	12
2	22	640	90	14
1	21	720	100	14
5	19	780	105	14·5
7	22	850	110	12
8	21	900	100	11
6	18	1060	120	11

TABLE III.

Semitendinosus with graded stimulation of musculo-cutaneous branch of ipsilateral peroneal nerve. Maximal reflex tension 1300 grm. in this preparation.
Constant stimulation of contralateral internal saphenous nerve at coil distance 27 cm.

Sequence of observations	Coil distance for excitatory afferent, cm.	*A*. Tension developed from excitation alone in grm.	*B*. Fall in tension during inhibitory stimulation in grm.	*B* expressed as percentage of *A*
13	15	90	0	0
12	14·7	130	0	0
11	14·3	130	15	11·5
10	14	270	90	33
9	13	490	115	23·5
The same as above except that coil distance for inhibitory nerve is 15 cm.				
8	14·5	130	0	0
7	14	150	0	0
6	13·5	320	160	50
5	13	410	250	61
4	12·5	540	220	41
3	12	620	180	29
2	11·5	680	0	0
1	11	780	0	0

These experiments (confirmed by four others) show that an inhibitory stimulus of constant intensity may have considerable effect in antagonising a reflex contraction of moderate size and yet be entirely ineffective against a minimal reflex contraction; and further, that up to a certain point, increasing intensity of the excitatory stimulus is accompanied by an increasing relative inhibition. If the argument detailed earlier is sound, both of these findings are explicable only on the hypothesis that the inhibition is falling on a restricted field of the motor-units composing the flexor half-centre, and that this inhibitory field is being overlapped more and more by the excitatory field as the size of the latter is increased.

We cannot claim to have been able to show this effect in all preparations, but this is not surprising since reasons have already been adduced for supposing that the inhibitory field engaged by maximal stimulation of a quite small afferent nerve may occupy the whole of one half-centre. Unless, therefore, the constant inhibitory stimulus is of sufficiently weak intensity, a positive result is not to be anticipated. In such cases 100 p.c. of small contractions is inhibited and the percentage steadily falls as the excitation is made stronger.

Other observations supporting the main conclusion are the following. In one preparation repeated submaximal stimuli of constant strength applied to the ipsilateral internal saphenous nerve evoked reflex rises of tension in semitendinosus of 220 to 300 grm. These could be inhibited

partially or completely by concurrent stimulation of the musculo-cutaneous branch of the contralateral peroneal nerve at all coil distances less than 22 cm. When a reflex response of approximately the same size (180 to 240 grm.) was evoked from the musculo-cutaneous branch of the ipsilateral peroneal nerve, the strength of the contralateral stimulation had to be increased to 15 cm. coil distance before any inhibition was manifest. In other preparations, a constant inhibitory stimulus has been found to produce very different degrees of relaxation when pitted against equal contractions evoked from different excitatory afferents. Thus in one experiment the same stimulus applied to the contralateral popliteal nerve caused no relaxation of 15 and 40 grm. of contraction evoked from the ipsilateral popliteal nerve, but inhibited 67 p.c. and 83 p.c. respectively of 15 and 30 grm. of contraction evoked from the musculo-cutaneous branch of the ipsilateral peroneal nerve.

The examples given in the last paragraph suggest the possibility that the inhibitory field of an afferent nerve of one limb may differ from the excitatory field of the corresponding nerve of the other limb more than from the excitatory field of some other nerve. There is nothing improbable in such a suggestion on *a priori* grounds, but our experiments are certainly not conclusive on the point. That the two fields from corresponding nerves are frequently not arranged on the same plan, is, however, borne out by Table IV, which shows that, in the preparation from which Table I was also constructed, the excitatory and inhibitory fields of the two popliteal nerves in one half-centre do not overlap at all when feeble stimuli are applied to these nerves.

TABLE IV.

Tibialis anticus with graded stimulation of ipsilateral popliteal nerve. Maximal reflex tension about 1500–2600 grm (6).

Constant stimulation of contralateral popliteal nerve at coil distance 14 cm.

Sequence of observations	Coil distance for excitatory afferent, cm.	*A*. Tension developed from excitation alone in grm.	*B*. Fall in tension during inhibitory stimulation in grm.	*B* expressed as percentage of *A*
1	18	15	?0	0
2	17	40	10	25
3	16	100	50	50
4	15	280	80	29
5	14	460	100	22
6	14	460	100	22
The same as above except that coil distance for inhibitory nerve is 18 cm.				
7	18	15	0	0
8	17	40	0	0
9	16	110	5	4·5
10	15	310	30	10

SUMMARY.

1. When an inhibitory stimulus of constant weak intensity is pitted against an ipsilateral flexion reflex of graded intensity, it frequently happens that the former is impotent against feeble intensities of the latter while producing well-marked relaxation of more powerful contractions. The percentage of the reflex that is inhibited (measured as isometric tension) increases with increasing strength of the excitatory reflex up to a point after which the percentage decreases. It is argued that these observations are only explicable on the assumption that the inhibition is falling on a fraction of the motor-units composing the flexor half-centre, and that the excitation is acting on a different fraction. The two "fields" overlap more and more as the size of the excitatory field increases.

2. Other observations are quoted in support of this main contention.

3. The excitatory field of a nerve of one hind limb is not necessarily composed of the same motor-units or arranged on the same plan as is the inhibitory field of the corresponding nerve of the opposite limb.

4. With maximal stimulation, the inhibitory field of a quite small afferent nerve probably includes the whole of one half-centre, thereby differing from the corresponding excitatory field.

APPENDIX.

By J. C. ECCLES.

For these and other experiments on the mammalian preparation the need was felt for some method of keeping the stimulated nerve under constant conditions throughout a long period of investigation. A modification of the Keith Lucas fluid electrode (12) was finally decided upon.

A glass tube of 0·9 cm. outside diameter and about 3 cm. long is constructed with a conical perforated partition as shown in the diagram. A rubber stopper closes the tube at each end, and through each stopper passes a wire to a flat silver plate lying on the inner surface of the stopper. Complete insulation of the stimulating leads is attained outside the stopper by a joint of Chatterton's compound, the wires themselves being rubber-sheathed.

The first step in placing the electrode in position is to dissect up the ligated and divided nerve for about 3 cm. The cotton of the ligature is then threaded through the glass tube from the upper end, and the nerve is drawn into the upper chamber. The cotton is temporarily fixed by

placing the lower stopper in position. The tube being held vertically, a fine pipette filled with physiological fluid at 38° C. is passed through the perforated partition, and the lower chamber is first filled, and then the upper by the overflow, which effectively removes air bubbles from the conically topped lower chamber. On withdrawal of the pipette the cotton emerging by the side of the lower stopper is pulled upon so as to draw the nerve through the central perforation, till its ligated end lies well within the lower chamber. The placing of the upper stopper in position requires great care in order to allow the nerve to rest uninjured in the passage lying between the opposed grooves in the stopper and upper end of the glass tube, and to exclude air bubbles as well. The space surrounding the nerve at its exit is then sealed with vaseline. The electrode, after having been placed so that the nerve lies in approximately its normal position, is fixed there either by clipping the skin tightly over it or by sewing it in place. Especial care must be taken to see that the nerve is not twisted or kinked either during or after the whole of the procedure detailed above.

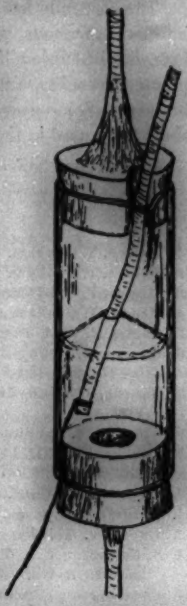

Fig. 1. Diagram of electrode showing nerve in position. The silver plate is seen on the upper surface of the lower stopper. The attachment of the rubber sheathed lead to the stopper is well seen in the upper one.

The fluid used in the electrode was originally Ringer Locke, but, as the excitability of the nerve was found to diminish after some hours in this medium, fresh defibrinated blood or blood serum was very advantageously substituted. A nerve under these conditions can be preserved in almost perfect condition for as long as 10 hours, and during the whole of that time the excitability remains extraordinarily constant. The deleterious effect of Ringer Locke is possibly attributable to the absence of colloid affecting the delicate surface membranes of the axis cylinders.

As the electrode is buried in the tissues, its temperature is maintained at the same value as the surrounding body parts, and is prevented from falling too low by sheathing the limb in cotton wool. The electrodes are non-polarisable if the silver plates inside the stoppers are coated with silver chloride; and the nerve is bathed by an optimal physiological

solution, which in addition has a large oxygen reserve for the use of the nerve if defibrinated blood is the fluid contained in the electrode.

The electrode has been successfully applied to the following nerves: internal saphenous, popliteal, peroneal or either one or both of its two branches just below the knee, posterior tibial or either one or both of its plantar branches, and the nerves to semitendinosus and to semimembranosus. Some nerves such as nerve to sartorius or to biceps are too short to be used in the present type of electrode.

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STUDIES IN MUSCULAR ACTIVITY. I. Determination of the rate of circulation of blood in man at work.

By A. V. BOCK, D. B. DILL and J. H. TALBOTT.

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The circulation rate in man may now be determined by three methods: the nitrous oxide method of Krogh and Lindhard(1), the ethyl iodide method of Henderson and Haggard(2) and the type of method first used by Christiansen, Douglas and Haldane(3).

This "Haldane" method, so-called, has proved most satisfactory in our hands. Three years ago Field, Bock, Gildea and Lathrop(4) described two modifications of it and reported a series of determinations on normal resting subjects. They demonstrated the relationship between the carbon dioxide pressure of arterial blood and that of Haldane-Priestley samples and thus helped to establish the trustworthiness of this method. Since that time the application of the Haldane method to exercising subjects has been under investigation in this laboratory. The method finally arrived at has involved experimental studies, at various metabolic levels, of the slope of the carbon dioxide dissociation curve of oxygenated blood for each subject; of the relation between the carbon dioxide pressure of arterial blood and that of alveolar air; and of methods for determining the carbon dioxide pressure of oxygenated venous blood.

The first of these problems has presented no great difficulty. It has been discussed to some extent already by Bock and his associates(5) and will be discussed more in detail in later papers of this series. It is enough to say here that up to a given metabolic level, fairly well defined for each individual, the carbon dioxide curve is unappreciably changed in height and slope. Beyond this metabolic level the height of the curve falls off rapidly and its slope changes slightly. On each subject studied during exercise samples of blood have been drawn at each of several metabolic levels and employed for orienting the carbon dioxide dissociation curves.

The technique for collecting during exercise samples of alveolar air which have the same carbon dioxide pressure as that of arterial blood has been described by Dill, Lawrence, Hurxthal and Bock(6). It was

found that samples of alveolar air collected automatically by the device of Lindhard(7), as modified by Henderson and Haggard(2), have the same carbon dioxide pressure as Haldane samples collected by giving the signal "Blow!" at the beginning of expiration. A series of arterial punctures were made on subjects working at levels ranging up to a consumption of two litres of oxygen per minute. Arterial blood was found to have the same carbon dioxide pressure as alveolar air samples collected as above described.

The experiments described in this paper are concerned with methods of determining rate of carbon dioxide production and of oxygen consumption and with the third problem mentioned above, viz. determination *in vivo* of carbon dioxide pressure in oxygenated venous blood.

The mixing chamber method of sampling expired air as illustrated in Fig. 1 has often been used by physiologists. It appears to be well adapted

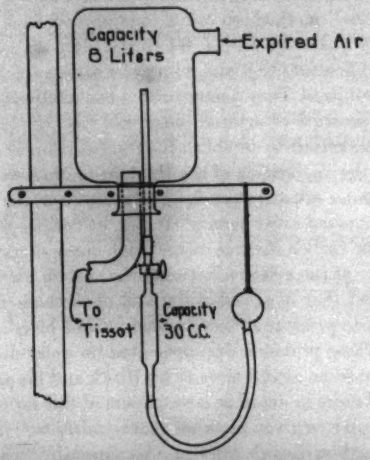

Fig. 1. Mixing chamber device for sampling expired air.

to exercise as it is often desirable to obtain samples at frequent intervals in order to follow change of state. We have been unable to find a thorough comparison of the mixing chamber method with the conventional Tissot method of sampling expired air in exercise. Accordingly, on several occasions samples taken simultaneously from Tissot and mixing chamber

have been compared. Thus, in an experiment on Dill, running on a treadmill, the expired air was led through the 8-litre mixing chamber into a 600-litre Tissot[1]. After flushing out the Tissot three times at one-minute intervals, the air expired in three minutes was collected. During this period two samples were drawn from the mixing chamber. Each of these samples was drawn over a thirty-seconds' period. At the end of the three-minute period the gas in the Tissot was mixed and two samples were drawn, one after pushing out a small amount of gas, another after nearly emptying the Tissot. The average ventilation rate was 73 litres per minute and the analytical results were as follows:

	CO_2 p.c.	O_2 p.c.	R.Q.
Mixing chamber, Sample 1	4·48	16·56	1·03
" " " 2	4·46	16·46	1·00
Tissot " 1	4·46	16·58	1·03
" " 2	4·48	16·40	0·98

A similar experiment on Talbott, running at a slower speed, is shown in Table I. Each period was five minutes and during each one sample was taken from the mixing chamber and another from the Tissot.

TABLE I.

Comparison of gas from mixing chamber and from Tissot spirometer.

Ventilation. Litres per min.	Mixing chamber CO_2 p.c.	Mixing chamber O_2 p.c.	Mixing chamber R.Q.	Tissot CO_2 p.c.	Tissot O_2 p.c.	Tissot R.Q.
56·4	3·52	17·10	$\frac{3·49}{3·93}=0·89$	3·48	17·12	$\frac{3·45}{3·90}=0·88$
56·9	3·51	17·09	$\frac{3·48}{3·94}=0·88$	3·49	17·10	$\frac{3·46}{3·93}=0·88$
57·2	3·67	17·00	$\frac{3·64}{4·01}=0·90$	3·57	17·02	$\frac{3·54}{3·99}=0·89$
56·2	3·54	17·14	$\frac{3·51}{3·86}=0·90$	3·65	16·97	$\frac{3·62}{4·05}=0·90$
58·5	3·56	17·10	$\frac{3·53}{3·91}=0·91$	3·49	17·07	$\frac{3·46}{3·90}=0·88$
54·9	3·77	16·72	$\frac{3·74}{4·32}=0·87$	3·71	16·82	$\frac{3·68}{4·22}=0·87$
58·9	3·43	17·01	$\frac{3·40}{4·05}=0·84$	3·52	17·06	$\frac{3·49}{3·95}=0·88$
Average	3·57	17·02	0·88	3·56	17·02	0·88

It is clear that representative samples can be obtained from the mixing chamber. Thus when the state of the subject is changing as at the beginning of work, or during recovery, the rate of change can be determined

[1] This Tissot and the special set of valves illustrated in Fig. 2 are manufactured by Warren E. Collins, 584, Huntington Avenue, Boston, Mass.

quite precisely by making frequent readings on the Tissot gauge and by drawing frequent samples from the mixing chamber.

The system of valves we have most commonly used is shown in Fig. 2. The subject inspires through Valve V_1 mounted in a glass housing,

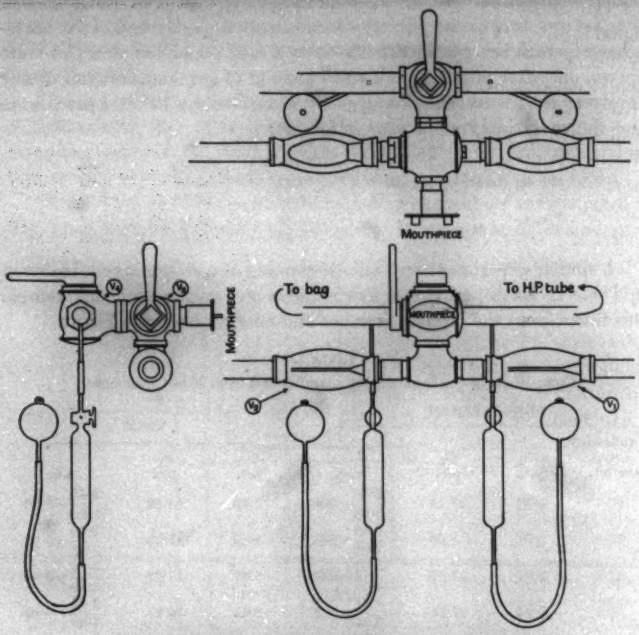

Fig. 2. Valve system used in blood-flow determination.

and expires through V_2 which is in a similar housing. The expired air passes through the 8-litre mixing chamber, Fig. 1, into a 600-litre gasometer. One person reads the spirometer gauge at frequent intervals and another draws samples slowly through a tube of capillary bore from the mixing chamber. Rate of oxygen consumption and of carbon dioxide production can be calculated from the composition of such a sample and from the corresponding rate of ventilation, corrected to standard conditions.

This device is suitable for rest or work except that when the ventila-

tion increases beyond 30 litres per minute, Valves V_1 and V_2 are replaced by similar valves of a larger size.

The procedure for collecting alveolar and "virtual venous" samples in exercise is as follows:

The subject breathes in through V_1 and out through V_2 until the signal is given. With V_4 open to Tube *HP*, V_3 is turned as the expiration begins and at the same instant the signal, "Blow!" is given. When the expiration is completed V_4 is turned to the bag and the signal "In!" is given. A rapid maximum inspiration is made while the operator holds a stop watch in his hand. After a suitable interval the signal "Out!" is given[1]. When it is completed V_3 is turned to its original position. The stopcocks on the two mercury sampling tubes are then opened and the samples of alveolar air and "virtual venous" air are collected. The "virtual venous" sample is collected, not from the bag but from the interposed brass tube as indicated in Fig. 2. It is our practice to place a rubber valve over the end of Tube *HP*, to prevent sucking back or entrance of air by diffusion. The whole process is repeated four times at intervals of $1\frac{1}{2}$ or 2 minutes. The bag at the start contains oxygen and enough carbon dioxide to exceed by 2 or 3 mm. the carbon dioxide pressure in oxygenated venous blood[2].

In such a manner samples and data are obtained from which blood flow can be calculated. The average carbon dioxide pressure in oxygenated, mixed venous blood and in arterial blood is applied to the carbon dioxide dissociation curve of oxygenated blood. The difference in corresponding contents, diminished by 0·2 volume p.c. (since in exercise,

[1] The original procedure of Field, Bock, Gildea and Lathrop(4) was similar to this except in three particulars: the signal was given at the end of expiration; the subject rebreathed the gas mixture and a sample was taken from the bag. We continue to give the signal at the end of expiration in resting subjects. It seems to be immaterial, however, whether a resting subject rebreathes or holds the gas mixture in the lungs. In exercise the holding method gives better evidence of an equilibrium than the rebreathing method.

[2] We have recently constructed a new apparatus which is a combination of that shown in Fig. 2 with the automatic box valve of Henderson and Haggard(3). Valves V_1 and V_2 with the connecting tee are replaced with the box valve. Valve V_3 and the tube connecting to the mouthpiece are unchanged.

This new apparatus may be used for total ventilation, automatic alveolar samples, Haldane-Priestley samples and "virtual venous" samples. We have used this apparatus at a metabolic rate of 3500 c.c. of oxygen used per minute, collecting expired air, automatic alveolar samples and "virtual venous" samples. For this purpose V_4 is opened to the bag, the subject blows with valves unchanged, turns V_3, inspires from the bag and after a suitable interval expires into the bag, and turns V_3 to its original position. With some practice an intelligent subject can perform these operations smoothly even at a metabolic rate which is 15 times the resting level.

as in rest, arterial blood is only about 95 p.c. saturated), divided into the rate of carbon dioxide production, determines the rate of blood flow.

We may now turn again to the evidence for the trustworthiness of our method for establishing the carbon dioxide pressure of venous blood. A series of experiments have been carried out on Bock and Dill precisely similar to the first of the methods described above except that the subject rode the ergometer for an hour or more and a great number of alveolar

TABLE II.

Subject, D.B.D., on October 7, 1927.

No.	Alveolar pCO_2 mm. Hg	Venous pCO_2 mm. Hg	ΔpCO_2 mm. Hg	Holding period seconds
1	38·4	56·9	18·5	5
2	—	68·8	29·0*	15
3	41·1	76·9	35·8	30
4	—	63·8	23·8	7
5	38·9	66·4	27·5	10
6	—	71·1	30·7	20
7	41·9	67·2	25·3	12
8	—	63·4	22·3	6
9	40·4	69·2	28·8	17
10	—	62·6	21·7	8
11	41·3	67·1	25·8	13
12	—	66·9	25·6	11
13	41·4	64·5	23·1	10
14	—	66·3	25·3	14
15	—	64·1	23·1	9
16	40·5	63·4	22·9	8
17	—	57·8	18·2	5
18	38·6	65·7	27·1	16
19	—	60·4	21·2	4
20	39·9	lost	—	—
Average	40·2			

Ventilation litres per min.	Composition of expired air: CO_2 p.c.	Composition of expired air: O_2 p.c.	R.Q.	CO_2 output c.c. per min.	O_2 used c.c. per min.
41·7	3·96	16·76	$\frac{3·93}{4·23}=0·93$	1650	1740
—	4·01	16·84	$\frac{3·98}{4·12}=0·96$	—	—

Calculation of blood flow.

	ΔpCO_2 during 6–9 second holding periods: mm. Hg	Slope on CO_2 dissociation curve	CO_2 transport vol.p.c.	Blood flow litres per min.
Three lowest: Nos. 8, 10, 16	22·3	0·40	8·72	$\frac{1650}{87·2}=18·9$
Two highest: Nos. 4, 15	23·5	—	9·20	$\frac{1650}{92·0}=17·9$

* An interpolated value for alveolar pCO_2 was employed in calculating alternate values ΔpCO_2.

and venous samples were taken. The holding periods varied in length from 3 seconds to as much as 50 seconds; the mixture in the bag was frequently renewed and the metabolic level varied in the series of experiments from rest to 2000 c.c. of oxygen per minute. These experiments were undertaken with the thought that after reaching a steady state a plateau might be found in the carbon dioxide pressures of our "virtual venous" samples. Such a plateau would suggest that an equilibrium can be established between gas mixture and venous blood before recirculation occurs.

Such an experiment is shown in detail in Table II. This experiment is chosen not because it is a "best" experiment but because it is average or poorer than average from our viewpoint. The calculation of the blood flow is also shown, based on venous samples collected after holding periods ranging from 6 to 9 seconds in length. The maximum is 18·4 litres per minute and the minimum 17·6 litres per minute. The essential data of this and eight other experiments on Dill are shown in Figs. 3

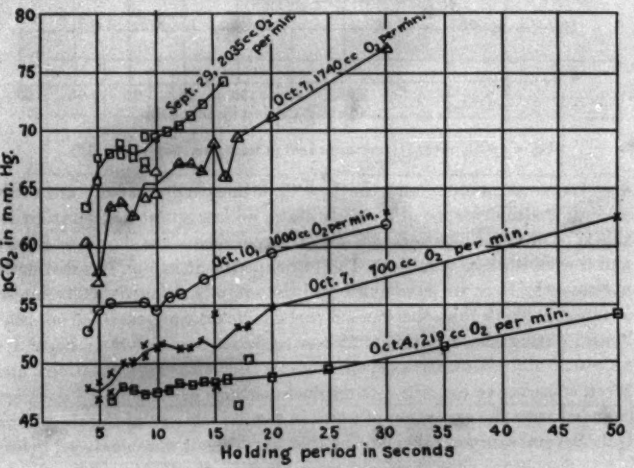

Fig. 3. pCO_2 of CO_2–O_2 mixtures held in the lungs. Subject, D.B.D.

and 4. In general it is not material whether one selects ΔpCO_2 or virtual venous pCO_2 to represent graphically as a function of the length of holding

period. The alveolar pCO_2 usually remained at the same level throughout the experiment, indicating a steady state. The holding periods were

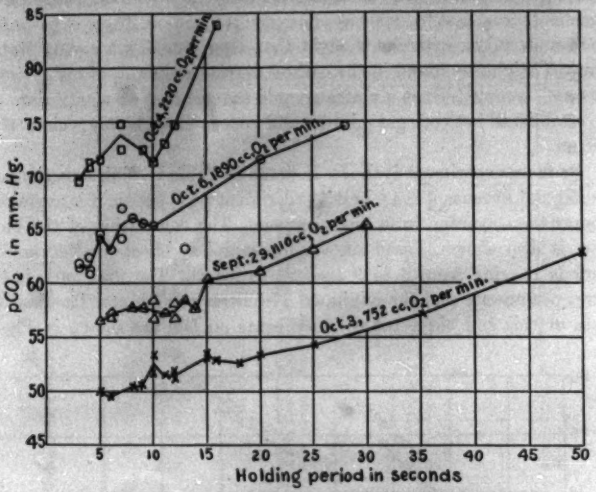

Fig. 4. pCO_2 of CO_2–O_2 mixtures held in the lungs. Subject, D.B.D.

also varied at random, and the fact that the results usually are fairly smooth is also evidence of a steady state, an important desideratum for this type of experiment. Seven similar experiments are shown in Figs. 5 and 6 with Bock as a subject. The top experiment in Fig. 6 is distinctly not smooth. Here we have numbered the samples chronologically, and it is fairly evident that the venous carbon dioxide pressure fell at least 3 mm. during the experiment. These experiments shown in Figs. 3–6 represent nine consecutive experiments on Dill, with no reservations, and seven consecutive experiments on Bock without exception. All data are included with the exception of a few in the experiment of October 10 on Dill. Several samples taken during that experiment were analysed before it was discovered that there was some alkali in the Haldane pipette. In experiments on both Dill and Bock the gas mixture was renewed after every fourth holding period until October 6. From that date it was renewed after each holding period.

No attempt has been made to draw smooth curves through these

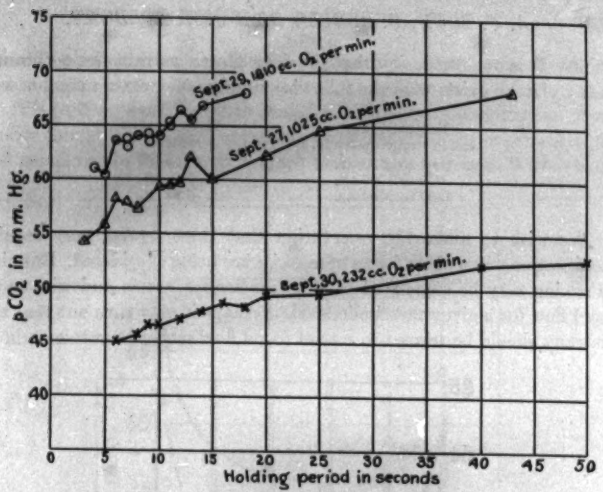

Fig. 5. pCO_2 of CO_2–O_2 mixtures held in the lungs. Subject, A.V.B.

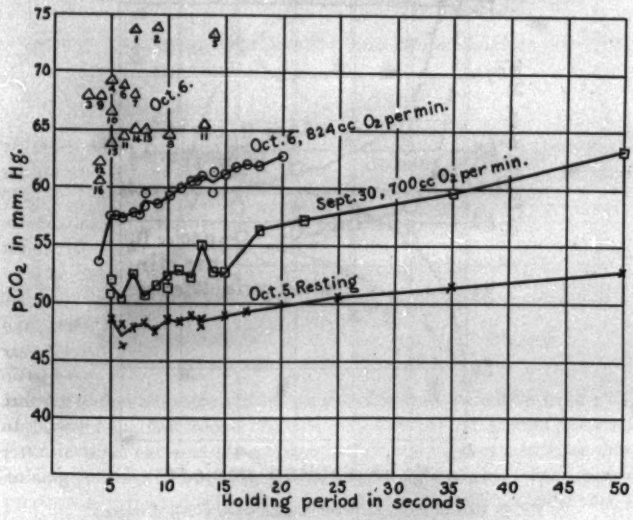

Fig. 6. pCO_2 of CO_2–O_2 mixtures held in the lungs. Subject, A.V.B.

points. It is our conclusion that the data shown warrant the conclusion that a plateau exists with the following limits:

Metabolic range c.c. of oxygen per min.	Plateau range seconds
Rest to 750 c.c.	12 to 16
750 to 1500 c.c.	9 to 12
1500 to 2200 c.c.	6 to 9

We wish to make the reservation that these figures may require modification when other forms of exercise are being considered. Running or rowing may be associated with better venous return and with more rapid flow for a given metabolic level. Perhaps it may turn out that the plateaux should be related to rate of blood flow rather than to metabolic

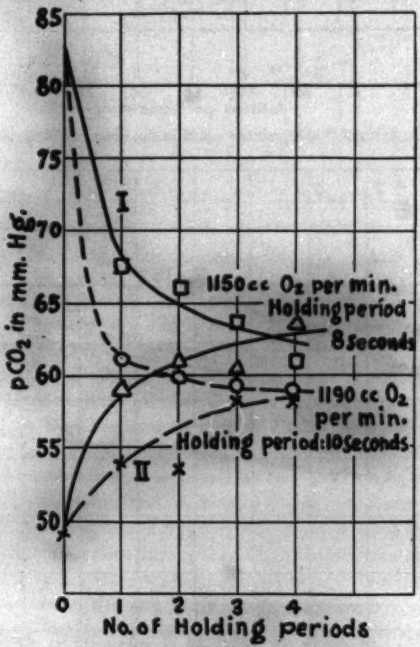

Fig. 7. Rate of approach to equilibrium when widely divergent gas mixtures are used. Subject, D.B.D.

rate. We plan on extending these experiments to other forms of exercise and to higher metabolic levels.

It is perhaps worth while to consider briefly one other question. How closely should the pressure in the bag be adjusted? Four experiments on Dill are shown in Figs. 7 and 8 which bear on this question. With a fixed

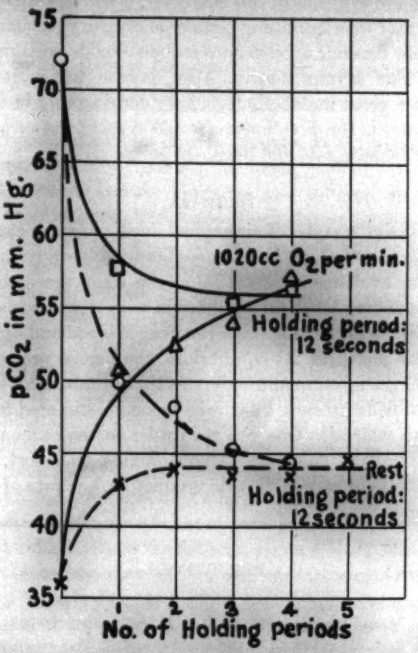

Fig. 8. Rate of approach to equilibrium when widely divergent gas mixtures are used. Subject, D.B.D.

holding period alternate virtual venous samples were drawn from a bag with very high, and from a bag with very low, carbon dioxide pressures. Even in these extreme cases the same pressure was reached after three holding periods. It is our practice to prepare a gas mixture with a partial pressure of carbon dioxide 2 or 3 mm. greater than the probable venous level and then to have the subject equilibrate this mixture in a pre-

liminary holding period. The subsequent holding periods are varied in length over the maximum range of the plateau known to characterise the grade of work being carried on. In this manner the reality of the plateau has been demonstrated on a great many occasions other than those described here.

In addition to evidence of this character, it will be seen in later papers of this series that with increasing metabolic rate, oxygen transport, which with blood flow depends upon venous carbon dioxide pressure, approaches a limiting value asymptotically. Also, oxygen transport and venous saturation at a given metabolic level vary considerably in different individuals and vary in the direction one would expect from such independent observations as pulse rate and degree of acidosis.

Summary.

Representative samples of air expired during exercise can be obtained from a mixing chamber. It is thus possible to follow any change of state precisely. Our evidence indicates that even when the metabolic rate is ten times the resting level, an equilibrium with regard to carbon dioxide can be established in the lungs between venous blood and certain oxygen-carbon dioxide mixtures. At equilibrium, the partial pressure of carbon dioxide in the gas mixture approximates that of returning venous blood. The carbon dioxide pressure in arterial blood is estimated from the composition of alveolar air. One can then refer to the carbon dioxide dissociation curves and calculate carbon dioxide transport. Having established the rate of carbon dioxide production, the rate of blood flow is simply calculated.

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STUDIES IN MUSCULAR ACTIVITY. II. A nomographic description of expired air.

By D. B. DILL[1] and A. FÖLLING.

(*From the Medical Laboratories of the Massachusetts General Hospital, Boston.*)

From the oxygen and carbon dioxide content of the expired air of an individual inspiring atmospheric air, the percentage of oxygen used, the percentage of carbon dioxide produced and in turn the respiratory quotient can be calculated. This tedious calculation has been simplified by Boothby and Sandiford(1). We have made a further simplification by the development of a D'Ocagne nomogram from which, knowing the composition of the expired air, one can read directly both the percentage of oxygen used and the respiratory quotient.

The construction of this type of nomogram is not difficult. Boothby and Sandiford have discussed the construction of their useful nomograms in detail. L. J. Henderson(2) has described the application of the D'Ocagne nomogram to the portrayal of the physicochemical interrelationships in the blood. While the nomogram shown in the figure is not of a complex character, we are giving empirical directions which will permit its accurate reproduction on a large scale.

Construction. On centimetre coordinate paper, draw X and Y axes as in a Cartesian chart. Draw the lines $x = 0$ and $x = 40$. These represent, respectively, the carbon dioxide line and the determined oxygen line. On the carbon dioxide line, mark 3·00 volumes p.c. at point (0, 15); 4·00 volumes p.c. at point (0, 35) and 5·00 volumes p.c. at point (0, 55). Divide into equal parts. On the determined oxygen line, mark 16·00 volumes p.c. at point (40, 5); 17·00 volumes p.c. at point (40, 25) and so on.

Between point (33·2, − 0·6) and point (33·07, 51·8), draw a straight line. This is the true oxygen line. Mark the first point 6·00 volumes p.c., the second, 2·00 volumes p.c. and divide into equal parts.

The respiratory quotient line is that straight line which joins the points (14·2, 8·0) and (23·1, 41·0). The exact points on this line can be

[1] National Research Fellow in Chemistry.

located most accurately by joining suitable points on the carbon dioxide line and on the true oxygen line. The intersection of such straight lines

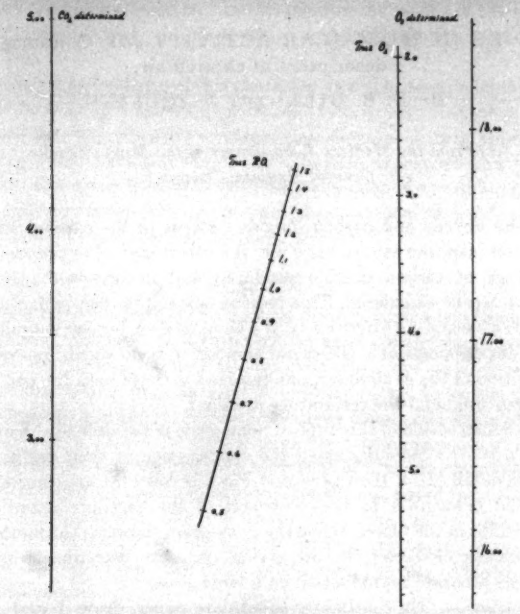

Fig. 1.

with the respiratory quotient line gives the respiratory quotient values according to the accompanying table.

Data for Calibrating the R.Q. Line.

Determined carbon dioxide p.c.	True oxygen p.c.	True R.Q.
4·53	3·0	1·5
4·23	3·0	1·4
4·58	3·5	1·3
4·23	3·5	1·2
3·88	3·5	1·1
4·03	4·0	1·0
3·63	4·0	0·9
4·03	5·0	0·8
3·53	5·0	0·7
3·03	5·0	0·6
2·53	5·0	0·5

We use a nomogram of the size described and have found it convenient and time-saving. One joins with a thread the percentage of carbon dioxide and of oxygen found in the expired air. The respiratory quotient and the percentage of oxygen used can be read directly with an error of less than 1 in 500.

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STUDIES IN MUSCULAR ACTIVITY. III. Dynamical changes occurring in man at work[1].

By A. V. BOCK, C. VANCAULAERT, D. B. DILL[2], A. FÖLLING and L. M. HURXTHAL.

(*From the Medical Laboratory of the Massachusetts General Hospital, Boston.*)

The object of this paper is to report a study of the physiological state of four normal male subjects during exercise on a stationary bicycle. In the interpretation of the data obtained consideration has been given to the character and severity of the work done by a given subject. An analysis of the results appears to show some of the adaptations which the organism as a whole may make as a result of physical training. The variations found in the different subjects are sufficiently great to indicate the range of individual reactions to the same type of muscular exercise, and for this reason throw some light on observations made in the past by others in which apparently contradictory results have been reported.

All of the subjects of this study were known to be in good health. One subject, DeMar, the Marathon runner, has maintained a state of physical training continuously for approximately 20 years. This athlete runs ten miles or more daily with few exceptions throughout the year. The subject C.V.C. had never had extended experience in any form of physical exercise. Both D.B.D. and A.V.B. have led sedentary lives but have been accustomed at times to periods of hard physical work. The former was a runner while in college. The study made in the case of DeMar covered a period of two weeks, the experiments being done before breakfast. The data in the case of C.V.C. were collected in a period of about six weeks, those on D.B.D. and A.V.B. in six months, the experiments generally being done 1–3 hours after breakfast.

Methods.

The work experiments were performed on a stationary bicycle equipped with a Prony brake. The periods of work lasted for 20 minutes or longer.

[1] The work on blood flow during exercise was begun in 1924 with Dr H. Field, Junr. It was continued in 1925 with Dr J. S. Lawrence and Dr L. M. Hurxthal. The form of the data now presented is the result of the cooperation of the present authors.

[2] National Research Fellow in Chemistry.

This form of exercise is not well adapted for work exceeding about nine times the resting metabolism if the work is done while the subject remains in a steady state, as was the case in these experiments. The nature of the exercise, involving chiefly the muscles of the legs, and the duration of the working period must be taken into account by those who wish to compare the data here presented with those obtained in other forms of exercise such as rowing or standing running, or with more severe grades of work done on a stationary bicycle for shorter periods of time. A steady state was reached in our subjects in from 2–4 minutes after work was begun. With the exception of C.V.C. the higher levels of work were not exhausting. Recovery periods following a few experiments during which the metabolic rate was about 2000 c.c. of oxygen per minute (subsequently referred to as M.R. 2000) showed a small oxygen debt. No recovery period is in hand in the case of C.V.C.

The blood flow method used in this study is described in Paper No. I of this series together with the technique of measuring the volume and collecting the samples of air expired. The pulse rate was counted by palpation of the apex beat. The blood pressure was read on a mercury manometer by the auscultatory method. Lactic acid in the blood was estimated by Clausen's (1) method. The carbon dioxide dissociation curves were obtained by equilibrating blood at 37·5° C., transferring the sample from the tonometer to a sampling tube in the water bath, and then analysing the blood for CO_2 with the Van Slyke-Neill manometric apparatus. The tension of the CO_2 in the tonometer was determined as previously described (2)[1]. Analyses of expired air were made with the Haldane portable apparatus, each apparatus being frequently checked by analysis of outdoor air. Our subjects rested on the saddle of the bicycle for at least ten minutes before observations in the resting state were begun.

Experimental data.

The data as found in individual experiments are comprised in Tables I–IV. Casual inspection of our results in a given subject will reveal a type of variation to be expected in a prolonged series of experiments of this character, irrespective of technical errors, since the response made by the organism at any given time may be influenced by many conditions often minor in nature. They serve to emphasise the difficulties encountered in any field of experimental biology when the attempt is made day after day to reproduce exactly the same conditions.

[1] A detailed description of the blood gas technique used in this laboratory will soon be published.

TABLE I. Subject, D.B.D. Age, 35; height, 180 cm.; weight, 71·5 kg.; vital capacity, 4700 c.c.

Date 1926	Pulse rate	Respiratory rate	Total ventilation l/min.	Oxygen used c.c./min.	CO_2 output c.c./min.	R.Q.	Alveolar pCO_2 mm. Hg	Oxygenated venous pCO_2 mm. Hg	CO_2 transport c.c./100 c.c. blood	Oxygen transport c.c./100 c.c. blood	Blood flow l/min.	Stroke vol. c.c.	Oxygen capacity Vol. p.c.	Venous saturation p.c.	Blood CO_2 at pCO_2 = 40 mm. Vol. p.c.
Oct. 8	70	10	6·1	250	190	0·76	36·3	44·7	3·56	4·69	5·3	76	18·5*	69·6	—
„ 9	76	15	12·0	578	482	0·83	36·9	51·1	6·12	7·36	7·9	104	18·5*	55·2	—
„ 11	77	12	5·8	243	190	0·78	36·6	45·5	3·77	4·82	5·0	65	18·5*	69·0	—
„ 15	100	16	21·4	1140	944	0·83	37·8	53·9	6·88	8·25	13·7	137	18·5*	50·4	—
„ 27	126	20	30·0	1590	1430	0·90	42·7	63·1	7·86	8·73	18·2	144	18·7*	48·3	—
„ 27	156	20	37·8	1750	1940	1·11	43·9	65·7	8·13	7·32	23·8	153	18·7*	55·8	—
„ 28	—	18	24·9	1290	1140	0·88	42·1	59·8	7·19	8·17	15·9	—	18·7*	51·2	—
Nov. 1	64	16	6·9	245	218	0·89	37·2	44·3	2·92	3·29	7·5	117	18·5*	77·2	—
„ 1	68	18	11·4	528	482	0·91	38·5	51·5	5·72	6·26	8·4	124	18·5*	61·2	—
„ 8	122	16	28·2	1420	1370	0·96	45·2	63·4	7·49	7·79	18·2	149	18·05	51·8	52·0
„ 10	120	17	31·3	1630	1480	0·91	41·2	63·0	8·70	9·10	17·0	141	18·74	46·4	48·0
„ 10	128	20	36·9	1680	1680	1·00	40·4	64·6	9·39	9·39	17·9	140	18·22	43·4	48·8
„ 12	103	16	23·1	1140	1070	0·94	41·0	62·5	8·59	9·14	12·4	120	18·66	46·0	48·0
„ 15	160	22	52·8	2300	2280	1·01	39·9	66·3	9·90	9·80	23·0	144	19·4*	44·5	—
„ 17	—	24	48·9	2250	2160	0·96	41·5	65·4	8·71	9·08	24·8	—	18·77	46·6	37·3
„ 22	160	24	53·4	2310	2420	1·05	42·2	67·5	9·03	8·60	26·8	167	19·78	51·5	34·8
Dec. 7	116	20	31·0	1590	1470	0·92	43·1	62·0	8·98	9·77	16·5	142	19·43	44·7	50·0
„ 10	162	24	50·9	2350	2350	1·00	41·9	70·9	10·92	10·92	21·5	133	20·55	41·8	40·0
„ 16	124	20	33·1	1790	1560	0·87	43·2	64·8	7·62	8·75	20·0	161	19·00	48·9	—
„ 27	160	24	51·6	2270	2360	1·04	44·8	69·4	9·79	9·42	24·1	151	19·43	46·4	40·0
1927															
Jan. 16	72	16	14·9	691	596	0·86	36·2	49·2	5·75	6·67	10·4	144	19·00	59·9	—
„ 25	158	30	54·6	2470	2350	0·95	40·1	65·8	9·50	10·00	24·6	156	20·59	46·4	47·9
Feb. 10	84	16	14·5	668	625	0·93	38·9	51·6	4·97	5·34	12·5	149	19·85	68·1	48·0
„ 10	90	18	16·7	821	728	0·89	39·5	53·4	5·46	6·14	13·4	149	19·85	64·1	48·0
„ 11	84	16	16·6	798	707	0·89	39·3	54·1	5·69	6·40	12·4	147	19·85	62·8	—
„ 21	144	21	39·6	1970	1880	0·96	41·9	63·7	9·08	9·46	20·8	144	20·42	48·6	46·7
„ 21	136	18	38·8	1820	1890	1·04	42·8	66·4	9·30	8·94	20·4	150	19·00	47·9	45·0
„ 21	140	22	39·3	2010	1930	0·96	41·2	64·4	9·78	10·20	19·5	139	20·42	45·0	46·7
„ 21	144	21	41·1	2020	1900	0·94	38·8	62·8	10·47	11·13	18·1	126	20·42	40·5	46·7
Mar. 30	152	20	41·2	1980	1820	0·92	43·2	66·9	9·56	10·39	19·0	125	20·70	44·7	45·6

* Assumed.

TABLE II. Subject, A.V.B. Age, 38; height, 177 cm.; weight, 71·5 kg.; vital capacity, 4200 c.c.

1926															
Oct. 27															

TABLE II. Subject, A.V.B. Age, 38; height, 177 cm.; weight, 71·5 kg.; vital capacity, 4200 c.c.

1926															
Oct. 27	—	—	8·6	366	272	0·74	32·7	42·8	4·65	6·27	5·84	—	21·0*	65·2	—
„ 27	120	21	39·9	1830	1630	0·89	37·1	61·8	10·49	11·80	15·5	129	22·0*	41·4	—
„ 28	121	18	28·3	1290	1220	0·95	39·2	58·6	7·60	8·03	16·1	133	21·0*	56·8	—
Nov. 4	126	24	39·6	1630	1650	1·01	—	—	—	—	—	—	—	—	—
„ 4	142	20	38·1	1580	1620	1·03	38·4	62·2	10·37	10·12	15·6	110	22·0*	49·0	—
„ 5	144	20	45·7	1960	1790	0·91	38·2	62·3	10·28	11·22	17·4	121	22·0*	44·0	—
„ 9	144	22	38·3	1640	1610	0·98	37·6	62·5	10·17	10·38	15·8	110	21·9	47·7	—
„ 11	110	20	27·5	1230	1180	0·96	38·4	61·9	9·69	10·11	12·1	110	21·9	48·9	44·8
Dec. 2	80	8	5·5	244	216	0·89	38·9	48·2	3·93	4·44	5·5	69	22·9	75·7	—
„ 4	88	7	5·8	253	204	0·81	34·1	44·1	4·12	5·14	4·9	56	21·7	71·4	47·5
„ 4	120	18	25·9	1210	1120	0·93	38·9	62·2	10·90	11·84	10·3	90	22·4	41·2	47·5
„ 8	90	8	5·8	275	208	0·76	34·2	44·2	4·50	5·90	4·6	51	21·0*	66·7	—
„ 9	114	15	23·8	1170	1030	0·88	39·6	60·4	8·49	9·54	11·9	105	21·0*	49·6	47·8
„ 15	120	22	39·2	1610	1620	1·00	40·4	64·9	9·49	9·49	17·1	142	22·0	51·8	—
„ 16	127	25	38·1	1760	1610	0·91	39·0	64·4	10·00	10·92	16·1	126	22·5	46·5	—
„ 16	132	—	48·6	2000	2000	1·00	37·9	65·0	10·70	10·70	18·7	142	22·5	47·5	—
„ 17	134	24	39·5	1680	1660	0·99	39·3	61·1	8·79	8·88	18·9	141	22·1	54·8	—
1927															
Jan. 7	160	20	42·8	1780	1840	1·03	38·2	65·2	10·71	10·39	17·2	108	22·9	49·7	—
„ 12	88	6	5·7	244	205	0·84	36·9	47·5	4·66	5·54	4·4	50	21·0*	68·7	—
„ 13	100	14	17·1	853	631	0·74	37·81	51·61	5·69	7·70	11·1	111	21·0*	58·4	—
„ 13	96	19	18·7	869	714	0·82	37·6	51·8	5·88	7·16	11·8	123	21·0	61·0	—
„ 19	148	27	62·0	2430	2400	0·99	37·1	65·9	11·30	11·41	21·2	143	23·2	46·0	38·4
Feb. 28	92	15	16·4	725	604	0·83	35·7	53·0	6·87	8·35	8·8	96	21·0*	55·2	—
Mar. 17	160	22	49·9	1920	1940	1·01	37·3	64·0	12·20	12·08	15·9	100	23·8	44·2	39·2
„ 22	160	25	50·6	2000	1830	0·92	37·0	62·1	10·30	11·26	17·7	111	22·9	45·9	41·2
„ 30	152	20	41·7	1980	1820	0·92	43·2	66·9	9·56	10·34	19·0	125	20·7	44·9	45·6

* Assumed.

TABLE III. Subject, C.V.C. Age, 25; height, 163 cm.; weight, 59 kg.; vital capacity, 3800 c.c.

1926															
Dec. 20	115	28	27·6	1230	1190	0·97	41·2	69·3	11·50	11·95	10·3	90	21·1	38·3	48·0
1927															
Apr. 18	96	14	6·75	267	227	0·85	40·6	52·2	4·81	5·64	4·7	49	21·2	68·3	—
„ 19	100	28	16·0	694	635	0·91	41·8	62·1	8·70	9·56	7·3	73	20·0*	47·2	—
„ 19	159	36	44·7	1880	1870	0·99	40·8	75·6	14·00	14·15	13·3	84	21·6	29·5	38·6
May 19	153	—	49·6	2030	2140	1·05	41·2	80·8	14·00	13·34	15·3	100	23·0	37·0	34·5
„ 25	101	26	18·4	788	742	0·94	41·4	63·5	9·00	9·58	8·2	81	20·0*	47·1	—
„ 25	124	—	28·5	1260	1220	0·97	41·2	68·5	10·80	11·14	11·3	91	21·3	42·6	45·7
„ 26	154	29	43·6	1900	1930	1·02	41·0	75·6	12·52	12·26	15·4	100	21·4	37·7	41·0
„ 26	88	15	5·8	229	173	0·76	—	—	—	—	—	—	—	—	—
„ 31	—	—	7·27	260	219	0·84	40·2	48·3	2·91	3·47	7·5	—	20·0*	77·7	—
„ 31	—	—	17·9	628	621	0·99	40·2	55·1	5·80	5·86	10·7	—	20·0*	65·7	—
„ 31	—	—	36·4	1450	1500	1·03	41·1	69·0	10·53	10·22	14·3	—	20·3	44·8	43·5

* Assumed.

TABLE IV. Subject, DeMar. Age, 39; height, 163 cm.; weight, 61·5 kg.; vital capacity, 5500 c.c.

Date 1927	Pulse rate	Respiratory rate	Total ventilation l/min.	Oxygen used c.c./min.	CO_2 output c.c./min.	R.Q.	Alveolar pCO_2 mm. Hg	Oxygenated venous pCO_2 mm. Hg	CO_2 transport c.c./100 c.c. blood	Oxygen transport c.c./100 c.c. blood	Blood flow l/min.	Stroke vol. c.c.	Oxygen capacity Vol. p.c.	Venous saturation p.c.	Blood CO_2 at pCO_2 = 40 mm. Vol. p.c.	Remarks
May 19	56	16	7·45	256	201	0·79	38·6	45·2	2·77	3·52	7·3	130	20·0*	77·4	—	Sitting
„ 19	120	30	48·1	2130	2080	0·98	42·0	63·7	8·70	8·87	23·8	198	21·5	53·7	46·5	Riding
„ 20	—	—	90·7	3519	3074	0·876	—	—	—	—	—	—	21·85	—	47·0	Running
„ 21	60	16	8·3	278	227	0·82	38·8	44·1	2·40	2·93	9·5	158	20·0*	80·3	—	Sitting
„ 21	—	—	35·4	1660	1430	0·86	40·8	60·2	8·20	9·54	17·5	—	21·0*	49·7	—	Riding
„ 27	82	23	22·4	903	918	1·02	41·8	56·1	5·77	5·66	15·9	194	20·0*	66·7	—	„
„ 27	127	36	48·1	2140	2040	0·95	42·5	64·3	8·70	9·16	23·5	185	21·5*	52·3	—	„
„ 28	62	16	8·67	251	217	0·86	36·0	43·6	3·36	3·91	6·5	105	20·0*	75·5	—	„
„ 28	120	36	45·7	2010	1910	0·95	40·8	65·4	9·80	10·32	20·1	167	21·0	45·8	47·0	Sitting
June 1	66	22	15·2	650	475	0·73	39·8	50·0	4·28	5·86	11·1	168	20·0*	65·7	—	Riding
„ 1	75	24	22·0	1020	827	0·81	40·5	53·5	5·38	6·64	15·3	204	20·0*	61·7	—	„
„ 2	112	33	44·3	2020	1850	0·92	42·2	64·6	8·90	9·67	20·8	186	21·5	49·8	48·0	„
1926																
May 18	54	14	9·11	334	310	0·93	37·8	47·0	3·97	4·27	7·8	144	20·2	73·8	—	Sitting
„ 19	46	12	5·08	211	185	0·88	43·1	49·1	2·20	2·50	8·4	183	20·0*	82·5	—	Basal; lying
„ 19	46	14	6·84	257	229	0·89	38·6	47·0	3·46	3·89	6·6	143	20·0*	75·6	—	Basal; sitting
„ 19	56	17	7·40	253	216	0·85	36·3	45·4	3·90	4·59	5·5	98	20·0*	72·0	—	Basal; standing
„ 20	80	20	21·0	870	777	0·89	42·6	—	—	—	—	—	—	—	—	Riding
„ 21	97	—	28·6	1250	1370	1·10	—	—	—	—	—	—	20·7	—	—	„
„ 22	118	26	38·5	1860	1830	0·98	—	—	—	—	—	—	20·9	—	—	„
„ 25	134	32	50·2	2450	2250	0·90	—	—	—	—	—	—	22·0	—	—	„
„ 26	134	30	56·4	2520	2490	0·99	—	—	—	—	—	—	21·8	—	—	„
June 9	—	—	91·8	3520	3460	0·98	—	—	—	—	—	—	—	—	—	Running

* Assumed.

In order to deal with the problems involved, we have taken the oxygen used per minute, the metabolic rate (M.R.) as the important independent variable. This may be regarded as a rough measure of the work done. The total ventilation, pulse rate, and stroke volume have been plotted against M.R. and smoothed curves drawn through the points, as in Fig. 1 from the data of Table I.

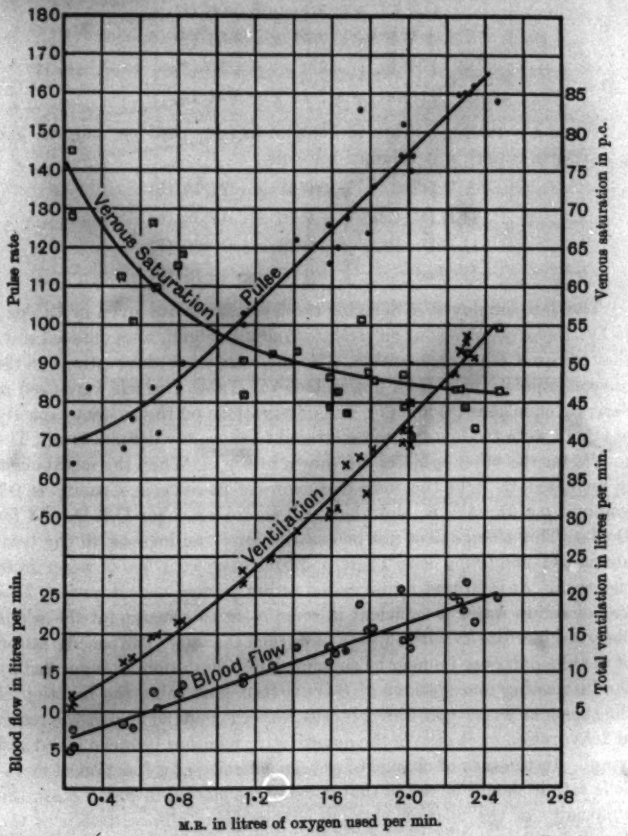

Fig. 1. Data of Table I, subject D.B.D., plotted as a function of metabolic rate.

For the data on blood flow and oxygen capacity the usual method was employed for setting up equations of straight line trend for rate of blood flow as a function of metabolic rate, and for oxygen capacity of the blood as a function of metabolic rate.

When x = metabolic rate in litres of oxygen used per minute, and y = rate of blood flow in litres per minute,

C.V.C. $y = 5{\cdot}1\ x + 5{\cdot}06$ (1)
A.V.B. $y = 7{\cdot}57\ x + 3{\cdot}54$ (2)
D.B.D. $y = 8{\cdot}2\ x + 4{\cdot}76$ (3)
DeMar $y = 7{\cdot}44\ x + 6{\cdot}31$ (4)

When x = metabolic rate in litres of oxygen used per minute, and y = oxygen capacity in volumes per cent.,

A.V.B. $y = 0{\cdot}37\ x + 21{\cdot}75$ (5)
D.B.D., 1926 $y = 0{\cdot}70\ x + 17{\cdot}38$ (6)
D.B.D., 1927 $y = 0{\cdot}72\ x + 19{\cdot}00$ (7)
DeMar $y = 0{\cdot}78\ x + 19{\cdot}78$ (8)

The data employed in deriving these equations are given in Tables I to IV. Some observations on DeMar standing or lying were omitted since they were not fairly comparable. Also there are a few observations on the oxygen capacity of D.B.D. not included in Table I which were used in deriving equations (6) and (7). The observations on the oxygen capacity of C.V.C. were too few and too scattered to show any definite trend. The results for the other subjects are shown in Fig. 2. When the metabolism is increased ten-fold the indicated increase in oxygen capacity is 0·7 volumes p.c. for A.V.B. and about 1·5 volumes p.c. for D.B.D. and for DeMar. This change does not necessarily imply an increase in the total amount of circulating cells. There is, during exercise, a loss of water from the serum, as indicated by increased serum protein concentration. This loss of serum water is sufficient in some cases to account for the whole observed increase in cell volume and oxygen capacity. The concentration of the blood in exercise may be an immediate adaptation, as suggested by the interesting observations of Barcroft(3) concerning the function of the spleen as a reservoir of red corpuscles, or it may be in part the result of dehydration or change in the quantitative relations between blood and lymph. In the case of change of oxygen capacity as a function of metabolic rate it should be noted that our data as plotted in Fig. 2 mask the uniformity of the relation through simultaneous representation of the day to day fluctuations in oxygen capacity during rest. A fair example of

the facts in a single experiment is given in Fig. 3. Further experiments dealing with this phenomenon are under way.

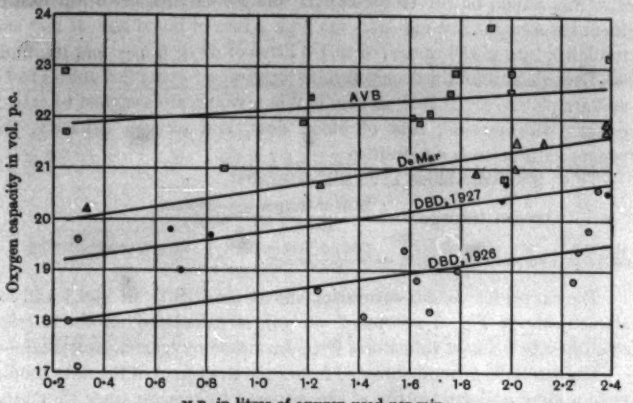

Fig. 2. Oxygen capacity of blood as a function of metabolic rate.

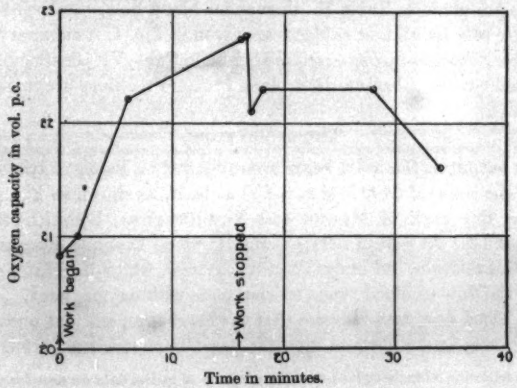

Fig. 3. Oxygen capacity in work and recovery. M.R. in work, 2200 c.c. per min.

We do not wish to imply that either rate of blood flow or oxygen capacity has a simple linear relation to metabolic rate. Having fitted the

best straight line to the points we are in a better position to decide whether or not the true relationship is linear. Inspection suggests that the blood flow line should be curved for D.B.D. and for DeMar, since for both of them the straight line indicates too high a rate of blood flow at rest and too low a rate in the range 0·6 to 1·0 litres of oxygen used per minute.

The calculated values for venous saturation are given in Tables I to IV and are plotted for D.B.D. in Fig. 1. When values are assigned to rate of oxygen consumption, rate of blood flow, and oxygen capacity, the venous saturation is determined.

Thus, if arterial blood is 95 p.c. saturated,

$$\text{Oxygen transport} = \frac{\text{Rate of oxygen consumption}}{\text{Rate of blood flow}},$$

and $$\text{Venous saturation} = \frac{\text{0·95 (oxygen capacity)} - \text{oxygen transport}}{\text{Oxygen capacity}}.$$

The curves for venous saturation shown for D.B.D. in Fig. 1 and for all subjects in Fig. 6 are based on values calculated as above from smoothed blood flow values and from smoothed oxygen capacity values.

The results in general obtained from such treatment of the data should yield a more uniform picture of the response to exercise since they tend to average fluctuations in the reactions of the body from day to day[1]. Summarised data taken from the average curves together with percentage changes are given in Table V. Curves for blood flow, total ventilation, and pulse rate for all four subjects are given in Fig. 4. A summary of the respiratory changes during exercise is given in Table VI, constructed from smoothed curves. Characteristic blood pressure readings are included in Table VII.

The blood flow.

The output of the heart bears approximately a linear relationship to M.R. in the interval 2400 > M.R. > 250 at least, as shown in Fig. 4. This confirms the work of Means and Newburgh(4), Boothby(5), and Lindhard(6). At rest in normal subjects blood flow and M.R. may not follow this relation, but under stress of exercise, which facilitates venous return, the flow of blood tends to keep pace with oxygen need.

The blood flow data indicate that the heart is an efficient pump even during severe work. The limitations imposed upon it when work is heavy

[1] The following references give preliminary reports of earlier data on exercise obtained prior to use of our present technique for determination of blood flow:

1. "Blood Flow During Rest and Exercise in Normal Man." A. V. Bock, L. M. Hurxthal, J. S. Lawrence, and D. B. Dill. Trans. Assoc. Amer. Phys. 41. p. 287. 1926.

2. *Lectures on Certain Aspects of Biochemistry.* H. H. Dale, J. C. Drummond, L. J. Henderson, and A. V. Hill. London: Univ. of London Press, p. 246. 1926.

A. Subject, [illegible].D.

Oxygen used	Pulse rate		Total ventilation		Blood flow		Stroke volume		Venous saturation	
			Absolute		Absolute		Absolute		Absolute	
c.c./min.	Absolute	Relative	l/min.	Relative	l/min.	Relative	c.c.	Relative	p.c.	Relative
250	70	100	6·5	100	6·0	100	85	100	75	100
500	74	106	10·5	161	8·2	136	110	129	63·5	85
750	84	120	15·0	230	10·3	171	123	145	55·0	73
1000	95	135	20·0	307	12·3	205	129	152	50·0	67
1250	108	154	25·0	384	14·5	241	134	157	47·5	63
1500	120	171	30·0	461	16·5	275	138	162	46·5	62
1750	132	188	36·0	553	18·7	310	141	166	46·5	62
2000	144	205	42·5	653	20·8	346	144	169	46·5	62
2250	156	222	49·3	757	22·9	381	147	173	46·5	62
2500	168	241	56·5	870	25·0	416	148	174	46·5	62
				B. Subject, A.V.B.						
250	88	100	5·5	100	5·0	100	57	100	72	100
500	93	106	9·9	180	6·9	138	74	129	63	87·5
750	99	115	15·0	272	8·9	178	90	158	58	80·5
1000	107	121	20·8	378	10·9	218	102	180	54	75·0
1250	115	130	27·5	500	12·9	258	112	196	51	71·0
1500	126	143	34·5	627	15·0	300	119	209	49	68·0
1750	138	157	42·0	763	16·9	338	122	214	47	65·0
2000	150	170	50·2	912	18·8	376	125	219	46	64·0
2250	163	185	58·8	1070	20·8	418	128	224	46	64·0
2500	177	201	67·5	1220	22·8	456	129	200	46	64·0
				C. Subject, C.V.C.						
250	90	100	6·8	100	5·5	100	60	100	73·0	100
500	96	107	12·0	176	7·0	127	73	121	57·0	78
750	102	113	17·5	257	8·0	145	80	133	49·0	67
1000	111	123	23·2	341	9·5	172	85	141	44·0	60
1250	122	135	29·2	430	10·8	196	88	146	41·0	56
1500	134	150	35·3	520	12·2	221	91	150	38·5	52
1750	146	162	41·6	612	13·5	244	92	152	37·0	50
2000	160	177	48·0	706	14·8	270	90	150	36·5	50
				D. Subject, DeMar.						
250	58	100	8·0	100	7·0	100	120	100	77	100
500	64	110	12·9	161	9·1	130	142	118	69	90·0
750	71	123	17·4	217	11·3	161	158	131	63	82·0
1000	79	136	22·3	279	13·4	191	170	141	58	75·0
1250	89	153	27·6	345	15·5	221	174	145	55	71·5
1500	98	170	33·3	415	17·6	251	180	150	52·5	70·0
1750	108	186	39·0	487	19·8	283	183	152	51·0	66·0
2000	118	203	44·8	560	21·9	313	186	155	50·5	65·5
2250	129	222	50·8	635	24·0	343	186	158	50·5	65·5

TABLE VI. Summary of respiratory changes during exercise.

Oxygen used c.c./min.	Respiratory rate	Total ventilation l/min.	Tidal air c.c.	Composition of expired air: Oxygen used true p.c.	Composition of expired air: Carbon dioxide added p.c.	R.Q.
			A. Subject, D.B.D.			
250	14	6·5	460	3·9	3·25	0·83
500	15	10·5	700	4·6	3·95	0·86
750	16	15·0	940	4·84	4·28	0·88
1000	17	20·0	1180	4·95	4·50	0·91
1250	18	25·0	1390	5·00	4·69	0·94
1500	19	30·0	1580	5·00	4·80	0·96
1750	20·5	36·0	1760	4·93	4·80	0·97
2000	22	42·5	1930	4·80	4·76	0·99
2250	23·5	49·3	2090	4·59	4·60	1·00
2500	25	56·5	2260	4·30	4·30	1·00
			B. Subject, A.V.B.			
250	7	5·5	787	4·3	3·60	0·82
500	11·5	9·9	860	4·53	3·83	0·85
750	15	15·0	1000	4·60	3·98	0·87
1000	18	20·8	1150	4·61	4·10	0·89
1250	19·5	27·5	1410	4·58	4·17	0·91
1500	21	34·5	1640	4·50	4·19	0·93
1750	23	42·0	1830	4·39	4·15	0·95
2000	25	50·2	2000	4·23	4·10	0·97
2250	27	58·8	2170	4·06	4·00	0·99
2500	28·5	67·5	2370	3·85	3·89	1·00
			C. Subject, C.V.C.			
250	16	6·8	425	3·95	3·10	0·80
500	21·5	12·0	560	4·14	3·65	0·88
750	25·5	17·5	690	4·28	3·92	0·92
1000	28·5	23·2	810	4·39	4·24	0·99
1250	30·5	29·2	960	4·45	4·45	1·00
1500	32·0	35·3	1100	4·45	4·50	1·01
1750	33	41·6	1260	4·39	4·45	1·01
2000	34	48·0	1410	4·25	4·32	1·02
			D. Subject, DeMar.			
250	16	8	500	3·40	2·70	0·79
500	18·5	12·9	700	3·70	3·12	0·84
750	20·5	17·4	870	3·98	3·50	0·88
1000	23	22·3	970	4·22	3·80	0·90
1250	25·5	27·6	1080	4·42	4·04	0·91
1500	28	33·3	1190	4·58	4·19	0·91
1750	31	39·0	1260	4·66	4·28	0·92
2000	34	44·8	1310	4·54	4·30	0·95
2250	36·5	50·8	1410	4·38	4·29	0·98
3295*	—	82·6	—	3·99	3·69	0·93
3519*	—	90·7	—	4·13	3·69	0·89

* Running.

TABLE VII. Blood pressure in exercise.

Experiment No.	Remarks	Oxygen used c.c./min.	Time from starting exercise minutes	Blood pressure Systolic mm. Hg	Blood pressure Diastolic mm. Hg	Pulse pressure mm. Hg
	A. Subject, DeMar.					
1	Rest	230	—	100	80	20
		1880	1	145	75	70
		2110	5	145	80	65
		1930	17	155	80	75
		1850	45	160	85	75
	Work stopped at 46 min.	—	46¼	140	80	60
		—	47	120	85	35
		—	48	102	85	17
		—	51	102	85	17
2	Rest	—	—	95	70	25
		—	1	105	70	35
		—	5	95	75	20
		878	10	105	70	35
		832	17	100	70	30
3		—	2	150	85	65
		2080	5	148	75	73
		2000	12	145	85	60
	Work stopped at 13 min.	—	13¼	120	80	40
		—	14	110	80	30
		—	14½	105	80	25
		—	15	100	80	20
		—	16	98	80	18
		—	18	90	70	20
	B. Subject, C.V.C.					
4	Rest	—	—	105	75	30
		—	1	140	70	70
		1177	4	150	65	85
		1275	8	165	65	100
		1175	15	165	70	95
	Work stopped at 16 min.	—	16¼	135	65	70
		—	17¼	110	80	30
		—	18¼	110	75	35
		—	20½	110	75	35
	C. Subject, A.F.					
5	Rest	—	—	140	80	60
		2050	2	230	90	140
		2300	14	260	90	170
	Work stopped at 16 min.	—	16¼	200	—	—
		—	17	160	80	80
		—	17¼	150	90	60
		—	18½	145	100	45

arise largely from conditions elsewhere in the body. At M.R. 2000, C.V.C. had approximately reached the maximum of effort of which he was capable. The other three subjects could work at this level for 20 minutes with comfort. In all four instances, judging from the blood flow curves alone, there is no evidence of failure of adaptation of cardiac output nor

is there a suggestion that a maximum blood flow has been reached. The significance of the differences in blood flow reactions in these subjects can

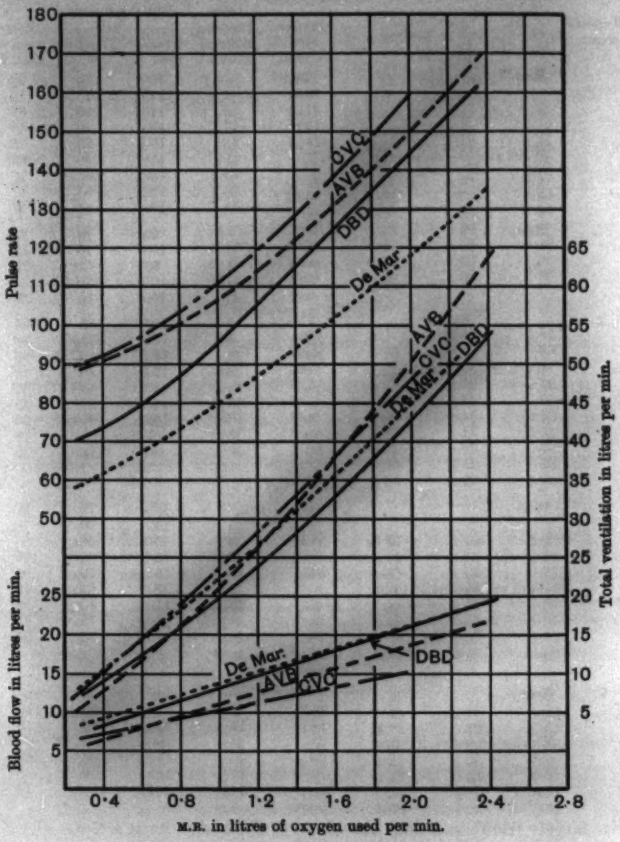

Fig. 4. Comparative changes in four subjects of blood flow, total ventilation and pulse rate as functions of metabolic rate.

be partially shown by an analysis of various factors entering into the problem of the work of the heart.

THE PULSE RATE AND STROKE VOLUME.

The curves for the pulse rates are shown in Fig. 4. Buchanan(7), Y. Henderson and associates(8), and others have referred to the slow pulse rates found in athletes. The curve for DeMar is an excellent example of this. Boothby(5) found a linear relation of the pulse rate to M.R. from rest to M.R. 900, but this does not hold during higher levels of work.

The pulse curves alone quite accurately depict the physical state of each of the four subjects. At M.R. 2000, DeMar's heart beats 118 per minute and that of C.V.C. 160 per minute. The slow pulse rate of the athlete appears to be directly related to the magnitude of the systolic output, or stroke volume, of his heart. Inspection of Fig. 5 shows that the

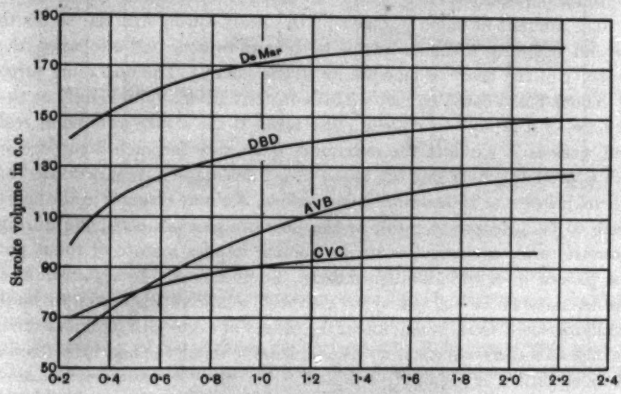

Fig. 5. Stroke volume as a function of metabolic rate.

stroke volume at rest of the athlete may be double that of the non-athletic subject. During work the stroke volume curve in the case of DeMar reaches an asymptotic level of 177 c.c. at M.R. 2000. In C.V.C. the resting stroke volume is about 70 c.c. and apparently reaches a maximum of 95 c.c. at M.R. 1700. The two intermediate curves of D.B.D. and A.V.B. probably more closely approximate the cardiac response of the average man of non-athletic pursuits.

Recently Y. Henderson, Haggard, and Dolley(8) have published data showing a uniform stroke volume in non-athletic subjects, but in the case of athletes they found an increase in stroke volume of 50 p.c. or more.

Douglas and Haldane(9) found a uniform stroke volume in one subject, C.G.D., during work. In our subjects, the athletic heart increased its stroke volume 26 p.c. and the non-athletic hearts showed increases of 36 p.c., 49 p.c., and 110 p.c. It is to be remembered that the stroke volume in our athlete at rest was double that of two of our subjects. Means and Newburgh(4) found an average stroke volume increase of about 70 c.c. at M.R. 1400 in their subject J.H.M. Krogh and Lindhard(10) found stroke volumes ranging from 70 c.c. to 150–160 c.c. If the stroke volume is actually a constant in non-athletic subjects, as Y. Henderson contends, the blood flow as determined during exercise for our non-athletic subjects would have to be in error by 35–55 p.c. This we think improbable.

The amount of blood pumped by the heart during exercise seems to depend not only upon an adequate venous return, but also upon the capacity of the heart to increase its stroke volume. The oncoming surge of venous blood could be met with a constant stroke volume only at the expense of a rapidly increasing pulse rate. If the stroke volume at rest and work is 70 c.c. and the maximum pulse rate for such a subject as C.V.C. were taken as 160, the blood would be completely unsaturated at a level below M.R. 2000, which is impossible. Volume changes in the heart seem to be a necessary result of the pressure changes occurring during exercise, while a changeless stroke volume implies a state of tonus not yet proved to exist in cardiac muscle. Furthermore, the organism as a whole, notable for its ability to establish adaptive responses to meet changing conditions, would, under the theory of a constant stroke volume, be deprived of a compensatory mechanism for which the heart, by reason of its structure, is peculiarly adapted.

The oxygen saturation of the mixed venous blood.

The average venous saturations for the range of work here considered are shown in the curves of Fig. 6. It will be seen that at various levels of metabolism in three of our subjects the venous oxygen saturation tends to reach a limiting value. As this limit is approached increase in blood flow is effected by increase in pulse rate and to some extent by slightly further increasing the stroke volume. In the subject C.V.C. a greater degree of acidosis at a given M.R. enables him to transport more oxygen than would otherwise be possible. In the case of DeMar, the lowest venous saturation is about 50 p.c., that of D.B.D., 46 p.c., that of A.V.B., 46 p.c., and that of C.V.C., 35 p.c. Taking into account the *p*H changes (to be referred to later) occurring at M.R. 2000 and applying the above

figures to appropriate oxygen dissociation curves, we can obtain the partial pressure of oxygen in the mixed venous blood for these values.

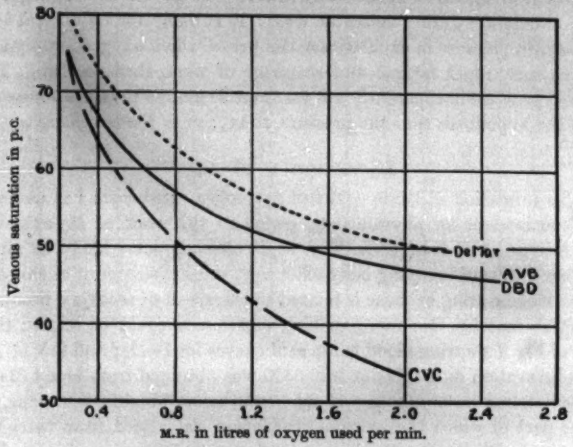

Fig. 6. Venous oxygen saturation as a function of metabolic rate.

They are respectively 24·5 mm., 24 mm., 24 mm., and 23 mm. The disparity in the figures for oxygen saturation largely disappears when the tensions of this gas are considered, owing to the effect of change in *p*H on the affinity of hæmoglobin for oxygen, the Bohr effect. The cost of this transaction, a shift in *p*H for maintenance of oxygen pressure in the blood, cannot be fully evaluated, but reference will again be made to it.

During rest the venous saturations have corresponding tensions for oxygen of 43 mm., 40 mm., 38·5 mm., and 39 mm. The quantities of oxygen transported in volumes per cent. are 3·0, 3·9, 4·7, and 4·0, and for work at M.R. 2000 they are 9·5, 9·75, 10·7, and 13·1. During exercise it is not known to what level oxygen tension may fall in the capillaries of active muscle. That level is probably lower than the above figures suggest since they represent a mixture of blood from active and relatively inactive parts of the body. When we take account, however, of the greatly increased blood flow, of the probability that most of this increase goes to the active muscles because of the local oxygen demand, and of a diffusion area enormously increased by the opening of many capillary pathways,

inactive during rest, it is probable that oxygen pressure in the capillaries will not fall greatly below the pressure found in the mixed venous blood. Haldane(11) and Henderson and Murray(12) discuss this aspect of the local circulation during muscular work. It is likely that a critical level for oxygen pressure in capillaries exists below which oxygen supply cannot be met, rapid fatigue and stoppage of work then resulting. This oxygen pressure is apparently not far from 20 mm., a pressure consistent with the hypothesis that the pressure of oxygen in tissue cells is zero.

Lactic acid in blood.

The formation of lactic acid from glycogen catabolism has assumed renewed interest for physiologists, owing to the work of Myerhof(13) and Hill(14). The level of lactic acid in blood appears likely to afford another means of studying body efficiency, since the amount of this acid in the blood during exercise is related to the speed of recovery processes in active muscles. No more striking illustration could be offered than that of Fig. 7 showing blood lactic acid curves for DeMar and C.V.C. The one observation on DeMar at M.R. 3500 was obtained from blood drawn immediately after stopping a 15-mile run (before breakfast), during the latter part of which the average ventilation, calculated from three observations with the Douglas bag method, was 90·7 litres per minute. The other observations were made from blood drawn while the subjects continued at work on the bicycle. The resynthesis of lactic acid in the case of DeMar keeps pace with its formation. Oxygen requirements are so fully met in his case as to maintain an internal environment at M.R. 3500 not unlike that of his resting state. Quite the opposite situation develops in a subject not accustomed to muscular exercise. In the case of C.V.C. the high level of lactic acid found during work reflects a failure of oxidation processes under conditions of stress.

The carbon dioxide content of the blood and pH_a.

We have previously published data from three subjects showing the degree of reduction in the carbon dioxide content of the blood during exercise(15), a phenomenon first found by Christiansen, Douglas and Haldane(16). The data given in Fig. 7 were collected in the course of experiments given in Tables III–IV. The plotted points represent in addition to lactic acid concentration, carbonic acid concentration in fully oxygenated blood when $pCO_2 = 40$ mm. Barr, Himwich and Green(17) concluded that the decrease in CO_2 of the blood did not parallel the rise in lactic acid. However, our results in the subject C.V.C., and recent

unpublished data indicate that changes in lactic acid and in carbonic acid are approximately reciprocal. The carbon dioxide level in the subjects

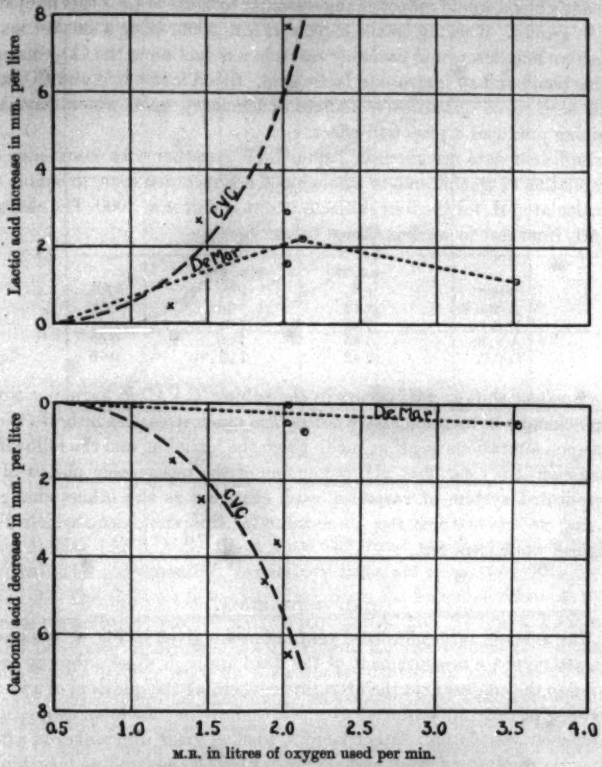

Fig. 7. Reciprocal relations of total carbonic acid and lactic acid in blood as a function of metabolic rate.

C.V.C. and A.V.B. begins to fall rapidly above M.R. 1000, and in the case of D.B.D. the normal level is held up to M.R. 1700. DeMar's blood remains normal throughout.

We can account for a constant level of CO_2 in blood during exercise up to M.R. 1000 on the ground of adequate oxygen supply. Up to that

point the three non-athletic subjects compare favourably with the winner of many Marathon races. At what point, while running, beyond M.R. 3500 DeMar's blood would reflect changes similar to those of the other subjects is not known. Working on the bicycle at M.R. 3000, using a smaller mass of active muscles, would probably result in a reduction in the CO_2 content of his blood and an increase in lactic acid. Blood lactic acid and CO_2 are thus seen to be quantitative indices of efficiency upon which physical training produces a profound effect.

Sufficient data are given in Tables I–IV, together with knowledge of the relation of true plasma to whole blood not recorded here, to enable us to calculate pH_s for the four subjects at rest and at M.R. 2000. The change in pH_s from rest to work is shown below:

Subject	M.R. 250 pH_s	M.R. 2000 pH_s	ΔpH_s
DeMar	7·42	7·40	0·02
D.B.D.	7·44	7·38	0·06
A.V.B.	7·45	7·36	0·09
C.V.C.	7·42	7·27	0·15

The greatest shift in pH_s occurs in the subject C.V.C. in harmony with other changes in function above noted. Too much stress has hitherto been put upon alterations in pH as such. From the foregoing and the following discussion it is clear that pH_s is but one of the measurable phases of a complicated system of variables, each changing as the others change, leaving no one of them free to account for the phenomena which distinguish work from rest.

Total ventilation.

The average curves for total ventilation are given in Fig. 4. We have not attempted a measurement of the dead space in these subjects and, owing to the confusion in the literature concerning the question of a fixed or changing dead space during exercise, we have not made an estimation of alveolar ventilation. Other factors, such as great differences in vital capacity, respiratory rates, and unknown variables controlling breathing, compel us to defer discussion of the response of breathing during exercise. Data are given in Table VI. The relations between change in alveolar pCO_2, pH, and volume of air breathed in the different subjects are so variable as to bring up again the question raised by Henderson (18) as to whether any single stimulus of the respiratory centre exists at all.

We will refer to one peculiarity of breathing, not hitherto discussed, in healthy subjects, *i.e.*, the varying manner in which the lung capacity

is brought into use. It will be seen in Fig. 8[1] that the figures for tidal air have comparable values at M.R. 2000 (total ventilation 42·5 and 50

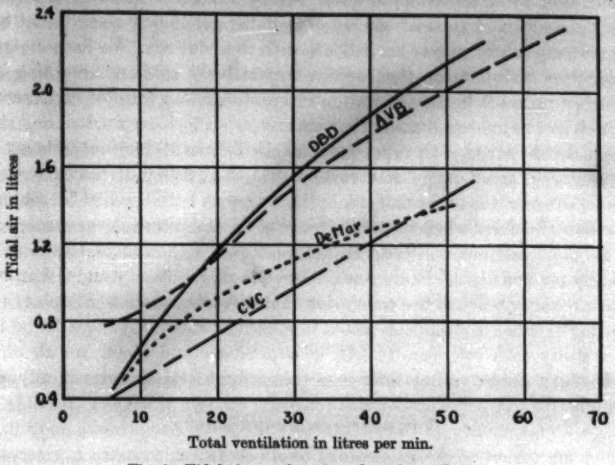

Fig. 8. Tidal air as a function of total ventilation.

litres) in the subjects D.B.D. and A.V.B., reaching about 2000 c.c., and for DeMar and C.V.C. about 1400 c.c. (total ventilation 44·8 and 48 litres). The corresponding respiratory rates are respectively 22, 25, 34, and 33. The tidal air in each subject bears the following relations to the vital capacity: 41 p.c., 47 p.c., 24 p.c., and 37 p.c. Sturgis, Peabody, and others (19) determined the maximum minute volume of pulmonary ventilation during work on a stationary bicycle in the case of twelve normal male subjects. They found an average respiratory rate of 35 per minute and tidal air averaging 33 p.c. of the vital capacity. The subject C.V.C. had probably approached the maximum ventilatory capacity of his lungs, judging from these data and the other reactions in this subject discussed above. DeMar, on the other hand, with a similar tidal air and respiratory rate, must have had remaining a large pulmonary reserve. During two running experiments, in which the pace set was about the average of that held during a Marathon race, we know that DeMar's pulmonary ventilation reached 90 litres a minute, a ventilation probably

[1] The S-shape of the curve for the subject A.V.B. is due to an unusually low respiratory rate while at rest.

20 or more litres below his maximum capacity while running. That his ventilation while at work on the bicycle was sufficient for his oxygen need is attested by all the available data. It may be argued that the work done was not sufficient to make demands upon his pulmonary reserve, but his rapid respiratory rate is an indication to the contrary. We have no explanation to offer for the rapid comparatively shallow breathing in DeMar, unless it be an adaptation in breathing to a form of exercise to which he was unaccustomed. The percentage of CO_2 in expired air and the virtual percentage of O_2 taken from inspired air in all four subjects show a relatively small range of variation, less than 0·75 p.c., indicating an equal efficiency as to gas transfer in the lung with both types of breathing, shallow and deep, when the tidal airs are of the magnitude here considered. This is of particular interest in the case of C.V.C. who had evidently nearly reached his maximum ventilation. Clark-Kennedy and Owen(20) have recently reached the conclusion that difficulty in elimination of CO_2 during exercise is a limiting factor in muscular effort. Changes found in our study with reference to CO_2 in expired air and blood are all of a secondary order, owing their rise primarily to the failure of oxygen supply.

The respiratory quotient.

A discussion of the respiratory quotient during exercise is reserved for the succeeding paper in this series. For present purposes it is sufficient for the reader to refer to the summarised data given in Table VI which show a steady rise in R.Q. during increasing amounts of work approaching or exceeding a quotient of 1. Data to be given in more detail reveal a quotient for the metabolism during many work experiments, both under fasting and non-fasting conditions, of 0·96 for a period lasting from 5 to 25 minutes. After this time there is a gradual fall in the value for the R.Q., presumably due to the increased utilisation of fat. For the period during which the supply of glycogen is abundant, the R.Q. tends to remain at a high level. This result conforms with the view of Furusawa(21) that carbohydrate in the form of glycogen affords the fuel from which muscles obtain the energy for contraction.

In the case of DeMar, the R.Q. is lower than that of any of the other subjects. This result is similar to corresponding data obtained by Benedict and Cathcart(22) in a trained bicycle rider. A possible explanation may be that the reserve of glycogen in the muscles of this subject (DeMar) is kept at a low level because of the daily habit of severe exercise necessitating in his case transformation and substitution of fat to meet the energy demands. On the other hand, the question arises as

to the effect upon the respiratory quotient of a change in the oxidation mechanism in the muscle cells. These cells will certainly participate in responsive changes to increased demands of function in a manner still unknown, but of a nature analogous to the increased function of the heart or lungs. Such changes, closely allied to the supply and pressure of oxygen, would lie in the direction of speeding up oxidation reactions, tending to prolong a steady state within the cell, as exercise proceeds, and make possible the completion of oxidations not commonly effected. Such a possibility of alteration in the intrinsic oxidative power of the living cells has been discussed by Hill and Lupton (23). They refer, for example, to possible changes of a chemical or colloidal nature in the surfaces which are the seat of the oxidation reaction, and to the possibility of increased quantities of such bodies as glutathione, capable of catalysing reactions. Phosphocreatine, discovered by Fiske and Subbarow (24), may be quantitatively increased.

Blood-pressure.

Blood-pressure determinations from a few experiments are recorded in Table VII. The rise in systolic pressure during work, together with its rapid fall upon cessation of work, have been observed by others. Lowsley (25) found an increase in both systolic and diastolic pressure during work on a stationary bicycle. Our data do not indicate any consistent change in diastolic pressure. In the case of DeMar, the systolic pressure does not increase greatly until work at M.R. 2000 is done, when it is comparable to that found in C.V.C. at one-half this rate. The subject A.F., not hitherto mentioned in this paper, is a physician (age 35, ht. 180 cm., wt. 68 kg.), who has not been permitted to undertake severe physical exercise since the age of 14. The extraordinary rise in systolic pressure in this subject is worthy of note. Knowledge of all the factors producing peripheral resistance to blood flow is not sufficient to account for such systolic pressure changes. The influence of respiratory movements on blood-pressure, for example, has never been clearly evaluated. With reference to this, we have found, during blood flow experiments, that while the subject holds the inspired mixture of O_2 and CO_2 in the lung, the systolic pressure drops from a previous resting level of 100 mm. of Hg to 75 mm., and during work from 165 mm. to 110 mm. Under these conditions the return of blood to the heart is momentarily reduced during the breath holding period. Increase of respiratory rate would tend greatly to increase the return of blood to the heart through the pumping action of the diaphragm and other muscles.

A varying elastic response of the aorta in different subjects may be a factor of great importance with reference to systolic blood-pressure, resistance being related to the manner in which the aorta accepts the load of blood discharged with each systole. We can conceive of such a function being highly developed in the case of DeMar and having a minimum development in a sedentary subject like A.F. An investigation pertaining to changes in the elasticity of the aorta is now in progress.

The relation between stroke volume and pulse pressure during rest is still a controversial subject. Many of our data, published and unpublished, concerning the effect of change of position, of adrenaline action, and of hyperthyroidism on blood flow, indicate a close parallelism between stroke volume and pulse pressure. During work the increase in systolic pressure must be partially due to increased stroke volume and increased volume of flow, but still other factors, especially in the untrained subject, must account for the greater part of the pressure change.

During our work experiments, we have seen no evidence of failure of the heart to pump forward the blood returning to the right heart, a condition tending to increase resistance in the arterioles. On the other hand, the greatly increased diffusion area resulting from opening of new capillary paths is very likely sufficient, at least in the trained subject, to take care of the increased blood flow through active muscles without greatly altering pressure conditions existing during rest. The dynamical problems involved in the interpretation of Krogh's discovery are especially interesting. In the untrained subject, the expansion of new capillary paths may be so limited in scope as to increase peripheral resistance to a notable degree.

The influence of stimuli from the cerebral cortex to the vasomotor centre is important at the beginning of work, as Krogh and Lindhard (26) showed, but we do not know to what extent these stimuli continue to influence blood-pressure as work is continued. Vaso-constriction of the splanchnic area during work may occur as Bainbridge (27) points out, but considering the fact that demand for increased blood flow is determined by the call for oxygen in the most active regions of the body, the total effect of splanchnic vaso-constriction on systemic blood-pressure must be small. It may be seen that fact and conjecture still leave much to be learned with reference to blood-pressure changes. Here, as elsewhere, the number of variables is too great to permit of a simple causal analysis.

Discussion.

No account has here been taken of numerous physicochemical changes occurring in the blood during exercise. For a statement of such of these as we have been able to measure, the reader is referred to a previous paper (15). It may be added that the saturation of arterial blood during work up to ten times the resting level has not been found to fall below 95 p.c., and may be 97 p.c. or 98 p.c., as Himwich and associates believe (28). This measurement has been made by us several times, but in the calculation of the venous saturation for the whole group of experiments we have taken the resting value of 95 p.c., excepting the experiments in which arterial saturation was determined.

From the standpoint of the efficiency of the body as a machine, final analysis of the data above presented shows the great advantage accruing to the organism as a result of physical training. The superiority of the athlete lies in his ability to meet the demand for oxygen, enabling him to maintain an internal environment varying within narrow limits only from the resting state. It is a well-established principle in physiology that function and use go hand in hand. The increased metabolism resulting from muscular exercise is met effectively only in the trained subject, through the coordination of a number of factors, the response, in general, being about what might be expected in a well-integrated system accustomed to such demands. Certain variables in the system have been measured and approximations can be made as to the relative importance of these. It is not possible, however, to estimate the rôle played by the nervous system, or by the glands of internal secretion. The actual extent of phenomena occurring in the capillary areas by means of which opening or closing of capillaries meet in varying degree the demand for diffusion surface also remains unknown.

Physical training increases the lung capacity, induces a slow pulse rate, increases the stroke volume of the heart, reduces systemic blood pressure during work, and probably greatly increases the capillary diffusion area in the muscles as it does in the lungs. Coordination of these and other unknown factors by the nervous system results in the provision of an adequate supply of oxygen to meet the needs of the organism up to levels of relatively severe work, with the maintenance of optimum conditions within the body for long periods of time. In the untrained subject, these mechanisms may be said to be relatively undeveloped. This statement applies less to the lungs than to the vascular system. Failure to meet oxygen in such a subject is reflected within a comparatively short

time in the accumulation of lactic acid, diminution of blood bicarbonate, a falling *p*H, and a tendency to reach maximum pulse and respiratory rates at levels of work that produce almost no embarrassment for the athlete.

Although little of a definite nature is known about the mechanism of cell oxidation, we believe that the capacity for exercise is bound up with changes in the oxidative processes within the cell. A search for the secret of such efficiency as we have shown to exist in the case of DeMar does not yield an answer in terms of increased efficiency of breathing or circulation alone. It appears to lie rather in the muscle cells themselves.

The limiting factor for exercise on a stationary bicycle is not the efficiency of the heart or lungs, but appears to be fatigue of the muscles of the legs. When work is done requiring much more than 2000 c.c. of oxygen per minute, the untrained subject can perform such effort only by going "all out," and then only for a few minutes. Increasing leg weakness, presumably related to accumulation of lactic acid, forces him to stop. For this reason, other forms of exercise requiring more widespread muscular action will lead to results differing from those reported in this paper.

Summary.

Some of the effects of physical training upon the efficiency of the body as a machine have been measured. The results indicate that training enables the organism to maintain an internal environment approaching that of the normal resting state even during severe work. While many adaptive responses have been considered, we believe changes in the oxidative mechanisms of the muscle cells, speeding up the rate of effective oxidation, to be the most striking compensatory adjustment established by training.

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STUDIES IN MUSCULAR ACTIVITY. IV. The "steady state" and the respiratory quotient during work.

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In view of the present conflict of observations and opinions concerning the respiratory quotient during work, the subject of this paper is approached by the authors with some trepidation. Questions pertaining to "basal" or "non-basal" conditions, duration, character and intensity of the work period, nature of the diet, experimental procedures, and the existence of a "steady state" have all been raised in connection with the interpretation of the respiratory quotient.

In the preceding papers of this series some account has been given of methods and of experiments conducted during work on a stationary bicycle. It is proposed now to examine in detail experiments demonstrating a "steady state," the condition in which, for the most part, our work has been done. In addition we present data concerning the respiratory quotient in a series of experiments in which this quantity has been determined at intervals up to one hour or more from the beginning of work. Some of these data have been dealt with in the preceding paper. The significance of the R.Q. is discussed in the light of the data given.

The "steady state."

The conditions determining the so-called "basal state" are now well understood. This represents the minimum metabolism necessary for the maintenance of the organism at rest and normal temperature. Similarly, during continuous muscular exercise a "steady state" is reached when the demand for oxygen is adequately met. Such a steady state implies a relatively constant total ventilation, elimination only of carbon dioxide produced in metabolism, steady pulse and respiratory rates and a constant internal environment. These conditions may be associated with an oxygen debt provided it is not cumulative but is chiefly acquired in the period from the beginning of work until the steady state is reached.

[1] National Research Fellow in Chemistry.

Much confusion has arisen in the past because these conditions have not been understood and their importance appreciated. Sometimes technical errors have also been responsible for data of misleading character. Furthermore, the response of the organism varies with the nature and intensity of the work done in such a manner as to make certain kinds of comparative studies wellnigh impossible. For example, a steady state cannot be maintained by the average man, not in training, for a continuous period of work on a stationary bicycle when the oxygen requirement of the work done exceeds about 2·0 litres a minute, but when larger muscle masses are in action in other forms of work this limit may be surpassed. Hill(1) reports instances of runners and oarsmen in whom the rate of oxygen intake may reach even 4·5 litres per minute during a steady state of work. Some of our recent experiments with subjects running on a treadmill indicate that an oxygen intake of 2·5 litres per minute can be continued for two hours during this form of exercise. It is obvious also that there is a time limit, varying from subject to subject, beyond which a steady state can no longer exist. The size of the subject, condition of physical training and nature of the previous diet are important variables.

For our present purpose two experiments performed on the same subject will illustrate what we believe to represent a steady state during work. The first experiment was done two hours after breakfast, the second before breakfast. The subject, D.B.D., is well accustomed to the use of the usual type of mouthpiece and nose clip used in respiratory work. In recent years his only experience of riding a bicycle has been that obtained on the stationary bicycle in the laboratory. He was a runner in college, but is not now in a state of physical training. His diet contained the usual amount of carbohydrate, fat, and protein. The technical procedures used have been discussed in Papers I and III of this series.

Exp. 1. February 21, 1927, non-basal. The data of this experiment are given in Table I and are presented graphically in Fig. 1. No observations were recorded until five minutes of the working period had elapsed, previous experiments having shown that this subject, at the level of work to be done, reaches a steady state within this time. Each figure given for the total ventilation was obtained from two-minute collection periods. In Fig. 1 these values are plotted at times corresponding to the middle of the collection period.

The trend of the data shown in Fig. 1 leaves little doubt that the subject was working well within his capacity to supply oxygen sufficient for his metabolic requirements. All of the other observations are in

TABLE I. Subject, D.B.D. two hours after breakfast on February 21, 1927. Age 35, height 180 cm., weight 71·5 kg.

Time min.	Pulse rate	Respiratory rate	Total ventilation l/min.	Composition of expired air: CO_2 added p.c.	Composition of expired air: O_2 used True p.c.	CO_2 output c.c./min.	O_2 used c.c./min.	R.Q.	Composition of alveolar air: pCO_2 mm. Hg	Composition of alveolar air: pO_2 mm. Hg	Blood flow l/min.	Venous saturation p.c.
5–7	136	18	38·8	4·88	4·68	1900	1820	1·04	42·8	102	—	—
8	—	—	—	—	—	—	—	—	—	—	20·4	48
10–12	144	20	39·3	4·90	5·11	1930	1970	0·98	42·5	101	—	—
22	—	—	—	—	—	—	—	—	41·2	100	—	—
24	—	—	—	—	—	—	—	—	—	—	19·8	43
25–27	140	22	39·7	4·59	5·07	1820	2010	0·91	40·9	100	—	—
30	—	—	—	—	—	—	—	—	—	—	19·0	44
40–42	144	21	38·4	4·76	5·03	1830	1930	0·95	41·9	99	—	—
44	—	—	—	—	—	—	—	—	—	—	22·3	51
58–60	144	21	41·1	4·63	4·92	1900	2020	0·94	38·8	103	—	—
62	—	—	—	—	—	—	—	—	—	—	20·7	47

TABLE II. Subject, D.B.D. on May 23, 1927, in the basal state.

Time mins.	Remarks	Total ventilation l/min.	Composition of expired air: CO_2 added p.c.	Composition of expired air: O_2 used p.c.	CO_2 output c.c./min.	O_2 used c.c./min.	R.Q.	Alveolar pCO_2 mm. Hg	Blood flow l/min.	Venous saturation p.c.
Resting on bicycle		6·15	2·75	3·68	169	226	0·75	—	—	—
0	Work began	—	—	—	—	—	—	—	—	—
1		34·1	4·50	5·55	1530	1890	0·81	—	—	—
2		41·7	4·73	4·51	1970	1880	1·05	—	—	—
3		47·3	4·42	4·70	2090	2220	0·94	—	—	—
4		48·3	4·13	4·51	2010	2180	0·92	—	—	—
8		48·3	4·17	4·46	2020	2160	0·94	—	—	—
10		—	—	—	—	—	—	36·7	—	—
19		—	—	—	—	—	—	—	23·1	48
25		45·8	4·16	4·44	1900	2030	0·94	36·5	—	—
31		—	—	—	—	—	—	—	21·0	46
35		—	—	—	—	—	—	35·7	21·2	46
37		47·7	4·06	4·28	1940	2040	0·95	—	—	—
38	Work stopped	—	—	—	—	—	—	—	—	—
38½	Recovery	37·9	3·41	2·61	1290	990	1·31	—	—	—
40		17·9	3·02	3·23	540	580	0·94	—	—	—
41		11·4	2·72	3·75	310	428	0·73	—	—	—
44		9·5	2·94	4·07	279	387	0·72	—	—	—
48		7·6	3·00	3·99	228	303	0·75	—	—	—
52		9·0	2·84	3·80	255	342	0·75	—	—	—
56		6·7	2·86	3·80	192	254	0·75	—	—	—

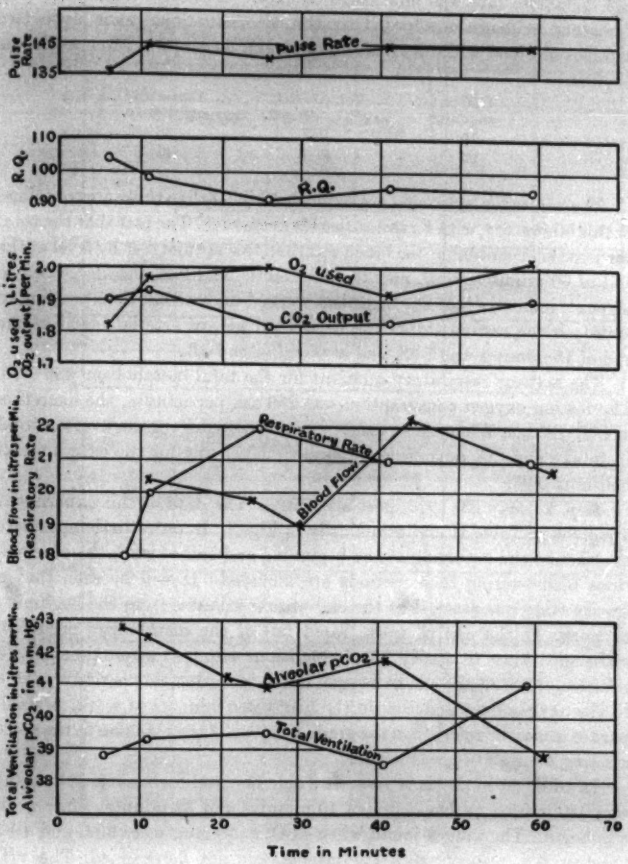
Pulse Rate
146
135
Pulse Rate
110
100
90
R.Q.
R.Q.
2.0
1.9
1.8
1.7
Litres per Min.
O2 used
CO2 output
O2 used
CO2 Output
22
21
20
19
18
Blood Flow in Litres per Min.
Respiratory Rate
Respiratory Rate
Blood Flow
43
42
41
40
39
38
Total Ventilation in Litres per Min.
Alveolar pCO2 in m.m. Hg.
Alveolar pCO2
Total Ventilation
0
10
20
30
40
50
60
70
Time in Minutes

Fig. 1.

keeping with an activity as steady as that obtained during rest, although the metabolic rate was nine times in excess of the resting state. Confirmatory evidence is afforded by the determinations made upon two samples of blood withdrawn 15 minutes and 60 minutes after work was begun:

Time in minutes	Vol. p.c. CO_2 $pCO_2 = 40$ mm.	Lactic acid mg./100 c.c.
15	45·2	55
60	47·5	43

The values for lactic acid are somewhat higher than those now obtained in this laboratory with Friedemann's method (2). The fact that the total carbonic acid content of the blood returned to a normal resting level at the end of 60 minutes work, and that the lactic acid had also decreased in amount, indicates that the organism worked under conditions tending to approach the resting state. The calculated pH$_s$ of arterial blood at the end of 15 minutes was 7·38, and at 60 minutes 7·43.

The average respiratory quotient for the total metabolism was 0·96. The resting oxygen consumption was 230 c.c. per minute, the excretion of CO_2, 188 c.c. When these values are subtracted from the average total O_2 intake and CO_2 output, it is found that the R.Q. for the excess metabolism is 0·97.

Exp. 2. May 23, 1927, post-absorptive. The data of this experiment are given in Table II and graphically in Fig. 2. In order to demonstrate the phenomena occurring at the beginning and end of work the observations made during these periods are included. It will be seen that a steady state was reached at the end of four minutes from the beginning of work. At the end of work, rapid fall of the metabolism occurred, although return to nearly basal conditions required approximately 15 minutes. Most of the excess oxygen taken in during this period was due to the oxygen debt acquired in the first three minutes of work. A small part of it may be referred to the gradual return of the machine to the state existing before work was begun.

In addition to the data given in Table II, total carbonic acid content was determined on blood drawn 10 minutes and 32 minutes after work was begun. The values found when $pCO_2 = 40$ mm. were 39·5 and 40·7 volumes p.c. respectively. Lactic acid was not determined. The pH$_s$ value of arterial blood, calculated from the alveolar CO_2 pressure and true plasma $BHCO_3$ was 7·35 in the first observation and 7·38 in the second. The importance of these observations lies in the fact that the relatively slight acidosis produced tended to diminish rather than increase in

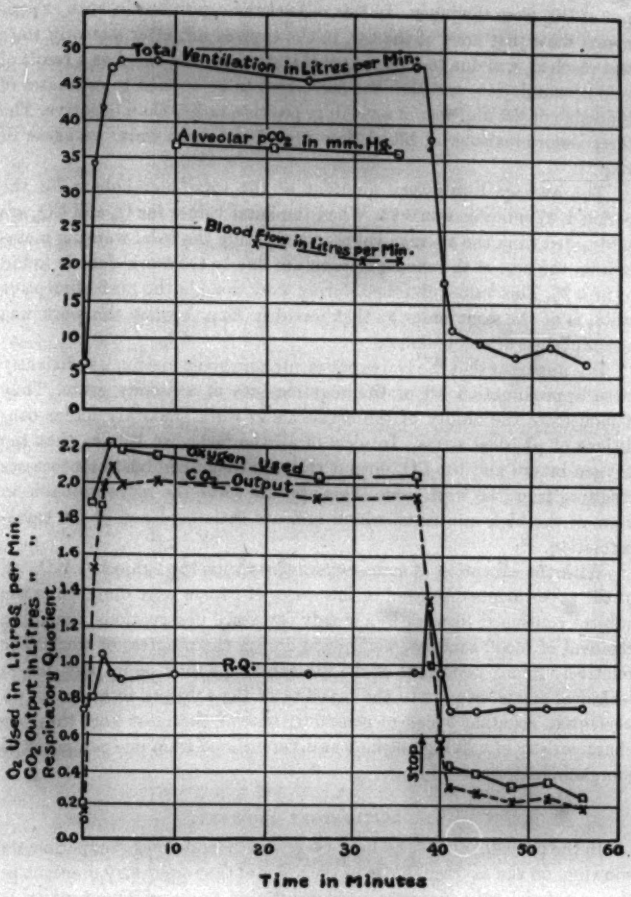
Total Ventilation in Litres per Min.
Alveolar pCO_2 in mm. Hg.
Blood Flow in Litres per Min.
Oxygen Used
CO_2 Output
R.Q.
Stop
O_2 Used in Litres per Min.
CO_2 Output in Litres " "
Respiratory Quotient
Time in Minutes

Fig. 2.

severity. This has an important bearing on the interpretation of the output of CO_2 from the lungs. In this respect the conditions in both experiments show that none of the CO_2 in the expired air, after a steady state was reached, was due to dumping of this gas from the blood as a result of acid accumulation, and that the correction to be applied to estimates of the value of the R.Q. was, if anything, positive rather than negative. The three determinations of blood flow are of the same order as those of Exp. 1.

The average respiratory quotient of the total metabolism for the period 4–37 minutes was 0·93. When the basal values for O_2 and CO_2 are subtracted from the average values constituting the total working metabolism, the R.Q. of the excess metabolism due to the work done is found to be 0·95. This value, obtained during work done in the post-absorptive state, is of the same order as that found in Exp. 1 when the work was accomplished after breakfast.

It is apparent that the two experiments here given meet in a sufficiently close approximation all of the requirements of a steady state. They demonstrate the ability of the organism to work smoothly under conditions of physical stress. In view of all the facts, we believe that the oxygen intake and the CO_2 output truly represent combustion processes resulting from the work done. This appears to be the more probable in view of the high quotients, which indicate little evidence of fat transformation.

With the exception of some experiments with the subject C.V.C., all of the experiments discussed in this series of papers were done while the subject continued to work in a steady state. All observations, including removal of blood samples, were made during the progress of work. The relatively minor variations of the measurements may be in part due to technical errors, in part to the inability of the subjects to maintain an absolutely constant speed of pedalling, but for the most part they are characteristic of a living machine as differentiated from one composed of inorganic materials.

The respiratory quotient.

In the preceding paper we have presented several series of experiments showing, on the average, a rise in the value of the respiratory quotient as the metabolic rate increases until a limiting value of about unity is reached in the hardest work which can be carried on in a steady state on the bicycle. In addition to this changing level of the quotient with increasing metabolic rate, we wish to call attention to the quotients

TABLE III. Subject, D.B.D.

Date 1926	Average oxygen used c.c./min.	Time periods during work: 4–9 min.	10–14 min.	15–19 min.	20–24 min.	25–29 min.	30–34 min.	35–39 min.	40–44 min.	45–49 min.	50–59 min.	60–69 min.
Oct. 28	2200	0·95	0·97	0·89	—	—	—	—	—	—	—	—
Nov. 4	1820	1·05	0·99	0·98	1·01	—	—	—	—	—	—	—
„ 10[1]	1630	0·91	0·91	—	0·90	—	—	—	—	—	—	—
„ 15	2310	1·12	—	1·06	—	1·02	0·97	0·97	—	—	—	—
„ 17	2250	0·97	—	0·95	0·95	—	—	—	—	—	—	—
„ 22	2310	1·07	1·01	1·03	—	—	—	—	—	—	—	—
Dec. 7	1590	—	0·94	0·90	—	0·91	0·93	—	—	—	—	—
„ 10	2360	1·04	1·02	1·01	—	—	—	—	—	—	—	—
„ 16	1790	0·83	0·84	0·90	—	0·89	—	—	—	—	—	—
1927												
Jan. 25	2470	0·96	0·94	—	0·92	—	—	—	—	—	—	—
Feb. 21	1960	1·04	0·96	—	—	0·91	—	—	0·95	—	—	0·94
Mar. 30	1960	0·95	0·96	—	0·98	—	—	—	—	0·91	0·88	0·89
May 23[1]	2100	0·94	—	—	—	0·94	—	0·95	—	—	—	—
		0·98	0·95	0·96	0·95	0·93	0·95	0·96	0·95	0·91	0·88	0·92

[1] Post-absorptive.

TABLE IV. Subject, A.V.B.

Date 1926	Average oxygen used c.c./min.	Time periods during work: 4–9 min.	10–14 min.	15–19 min.	20–24 min.	25–29 min.	30–34 min.	35–39 min.	40–44 min.
Nov. 1	1650	1·00	—	1·01	—	—	—	—	—
„ 4	1580	1·00	—	—	1·01	—	—	—	—
„ 5	1960	—	0·92	0·92	—	0·91	—	—	—
Dec. 15	1575	1·04	—	0·99	1·02	—	—	—	—
„ 16	1760	—	0·93	—	0·89	0·93	—	—	—
„ 16	2000	0·99	0·99	0·99	—	—	—	—	—
„ 17	1720	1·06	—	0·97	1·00	—	—	—	—
1927									
Jan. 7	1780	1·01	1·04	1·01	0·95	—	—	—	—
„ 19	2400	0·99	0·99	—	—	—	—	—	—
„ 21	2100	1·02	—	1·02	—	—	—	—	—
„ 26	2080	1·04	1·00	0·94	—	—	—	—	—
Mar. 17	1980	1·03	1·01	—	0·97	1·03	—	—	—
„ 18	2410	1·09	1·06	—	—	—	—	—	—
„ 21	2170	1·04	0·96	—	—	—	—	—	—
„ 22	2000	0·94	—	0·90	0·90	—	—	0·90	0·88
May 17[1]	2050	1·04	—	1·00	0·93	0·94	—	—	—
		1·02	0·99	0·98	0·96	0·95	—	0·90	0·88

[1] Post-absorptive.

Table V. Subject, C.V.C.

Date 1926	Average oxygen used c.c./min.	Time periods during work: 6–9 min.	10–14 min.	15–19 min.	20–24 min.	25–29 min.	30–34 min.	35–39 min.	40–44 min.	45–49 min.
Dec. 20	1230	0·95	—	—	0·95	—	—	0·97	—	0·96
1927										
Apr. 19	1880	—	1·03	—	0·96	—	—	—	—	—
May 19	2030	—	1·00	1·08	—	—	—	—	—	—
„ 25	1260	1·00	—	1·05	0·92	—	—	—	—	—
„ 26	1900	1·04	1·01	0·94	—	—	—	—	—	—
„ 31	1450	0·97	1·03	1·02	—	—	—	—	—	—
		0·99	1·02	1·02	0·96	—	—	0·97	—	0·96

TABLE VI. Subject, DeMar.

Date 1927	Average oxygen used[1] c.c./min.	Time periods during work: 3–9 min.	10–14 min.	15–19 min.
May 19	2130	1·03	0·95	0·93
„ 27	2100	1·00	0·96	0·93
„ 28	2010	0·94	0·94	0·92
June 2	2020	0·90	0·88	—
		0·96	0·93	0·93

[1] All post-absorptive.

obtained in our four subjects when heavy work was done. In Tables III, IV, V, and VI we have assembled the respiratory quotients obtained in a series of such experiments in each of which several determinations of

the quotient were made as the work progressed. In the case of the subjects D.B.D. and A.V.B., when the oxygen used is below 1500 c.c. per minute, the value of the R.Q. tends to remain below 0·95. Beyond this metabolic rate, as shown in Tables III and IV, the quotients tend to approach unity. It will be noted that there is a gradual fall in the ratio below 0·95 after work has progressed for 45 minutes in the case of D.B.D., and sometime after 30 minutes in the subject A.V.B. After these periods of time lower levels for the quotient are found. In the subject C.V.C. a few experiments not included in Table V have shown quotients above 0·95 when the oxygen used per minute did not exceed 1000 c.c. For work beyond this level the quotient becomes approximately unity. DeMar, on the other hand, unlike the other subjects, maintains a relatively low quotient until the work done requires an oxygen intake greater than 2000 c.c. per minute. These differences with respect to the respiratory quotient are in line with all the other observations characterising the four subjects noted in the preceding paper.

Discussion.

There has been a widespread opinion that the respiratory quotient cannot be taken as an indication of combustion processes in the body unless it is obtained by the chamber method or unless the experiments are conducted for a long period of time (3). If experimental conditions are not properly controlled, and account taken of the capacity of the subject to do the work set for him to do, there is no doubt that the quotients obtained by indirect methods are valueless. Over-ventilation due to unfamiliarity with breathing apparatus, and increasing acidosis due to accumulating oxygen debt, for example, produce a disturbance in CO_2 output that has no meaning in respect to combustion. If, however, the conditions of a steady state are realised, and more especially if this state is secured at a level of relatively severe work, the organism as a whole appears to react less to minor disturbing influences than is the case at rest. This is a very general physiological principle. Under such conditions the output of CO_2 bears a direct relation to the utilisation of oxygen. The respiratory quotient may therefore be taken as a true one.

In view of our experimental data and taking into consideration the nature of the organism as a whole, we adhere to the following hypothesis with reference to the significance of the respiratory quotient as a means of interpreting the processes of catabolism. The primary source of energy for muscular contraction in early stages of work is carbohydrate, as many others now believe. In the average man during moderate work glycogen

is utilised, but at the same time the velocity of reactions involving its synthesis from fat or other processes by which other sources of energy may be utilised nearly keep pace with the rate of glycogen utilisation. The resultant R.Q. will rise only slightly above the resting level. Given more severe work to do, muscle utilises stored glycogen, the utilisation of which may be considered to occur with a velocity of the first order, and the resulting quotient approaches unity. Time relations are now such that the conversion of fat at a velocity, let us say of the second order, has no appreciable effect upon the respiratory quotient. The quotient will continue to remain high until the store of glycogen is considerably lowered. That depletion of readily available glycogen results in speeding up the reaction Fat → Glycogen, or whatever other processes lead to the utilisation of other substances, seems likely. The quotient will then reflect this reaction.

The strongest argument in favour of the view that glycogen is the invariable source of muscular energy seems to us to depend upon the consideration that the intrinsic physicochemical processes involved in muscular contraction are always the same. Hence it seems probable that whatever different sources may provide the energy of muscular contraction, the chemical processes through which all substances pass must be in part common to all of them. It may perhaps be argued, even in face of the evidence of the importance of glycogen in muscular contraction, that this path may not include glycogen, but at least it must include some substance into which glycogen passes very readily.

Such an hypothesis explains the low quotients obtained when the work load upon the organism is light. It seems to account for the great differences observed in different individuals, especially if we admit that physical training enlarges the responses to exercise of the organism as a whole. Glycogen utilisation in an under-trained subject like C.V.C. overshadows fat transformation at levels of work considered very light for a subject like DeMar. In DeMar all oxidation processes appear to be speeded up so that no great change occurs in the quotient until very heavy work is done. It is assumed, other things being equal, that the rate of cellular metabolism may be altered through training. The theory proposed implies a change in the character of the catabolism when heavy work is done, as was suggested by Benedict and Cathcart(4). The observations of these authors are of especial interest in connection with the discussion in hand. In their subject, M.A.M., a trained bicycle rider, an extraordinary number of observations were made during work on a stationary bicycle. This subject failed to show striking changes in the

respiratory quotient during work, but in the experiments in which the work was heavy the highest quotients were obtained. In their untrained subjects, H.L.H. and E.P.C., quotients similar to those reported in this paper were found.

To return to other aspects of the problem, we find that in the past emphasis has been placed upon the question of consumption of fuel from food ingested before the experiment. Experiments 1 and 2 on the subject D.B.D., with and without breakfast, show quotients for the excess metabolism of 0·97 and 0·95, respectively. A similar result was obtained in an experiment on A.V.B. on May 17 in the post-absorptive state. In this experiment the average oxygen consumption during the steady state was 2100 c.c. per minute. The quotient of the excess metabolism was unity. All other experiments on this subject, done after breakfast, with heavy work have quotients of about this order. These results suggest that the presence of food in the gastro-intestinal tract has little to do with the source of the energy utilised *during severe muscular work*. On this question Cathcart and Markowitz(3) have the following to say: "*We come back to the old quarrel between Bonn and Munich. Is it necessary or not that the material ingested become an integral part of the living protoplasm before it is utilised? No unequivocal answer can be given to this question, but we are of the opinion, contrary to many workers, that the balance of evidence is in favour of the view that the material to be utilised must become in some way an integral part of the protoplasm of living matter before it can undergo either anabolism or catabolism.*" More experiments are under way looking toward a solution of this problem.

Another factor bearing on the respiratory quotient during exercise is the nature of the previous diet. On a high carbohydrate diet Benedict and Cathcart found higher values for the quotient in the subject M.A.M. than were obtained during a period of high fat diet. A large supply of stored glycogen should theoretically yield a high quotient during prolonged work of some severity, particularly in an untrained subject. On the other hand, Furusawa(5) in a series of standing running experiments secured quotients of unity during both high carbohydrate and high fat diets. These results we should now anticipate because the work done was severe and the experiments were concluded before sufficient time had elapsed to develop evidence of gylcogen exhaustion. It is likely that during prolonged experiments the quotient would fall in value in both series, but in a shorter time in the case of experiments done on a high fat diet.

To sum up, we believe, both on particular and general grounds, that

glycogen or some substance very closely related to it in normal metabolism is the immediate source of the energy of muscular contraction. The problem is too complicated to permit of any simple form of demonstration, but the evidence in favour of this view cannot be denied. We suggest that physical training produces a profound effect in altering the level of metabolism at which the phenomenon of a respiratory quotient approaching unity appears, and that for any subject the severity of the work done together with its duration in a steady state are the chief criteria to be taken into account. In untrained subjects, as the working period progresses, the quotient tends to fall. The hypothesis we have presented to explain these facts is useful as a working principle, but much future work remains to be done.

Summary.

The conditions defining the "steady state" of muscular exercise have been described and typical experiments illustrating this condition have been presented. During severe work on a stationary bicycle the R.Q. was found to be of the same order of magnitude for work done in both absorptive and post-absorptive states.

An hypothesis concerning the source of muscular energy and the varying manner in which different individuals supply the demand for energy has been set forth as a useful working principle.

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SUBLIMINAL FRINGE IN SPINAL FLEXION.

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(*From the Laboratory of Physiology, Oxford.*)

With flexor muscles, in the spinal flexion-reflex (hind limb, cat), it was found that stimulation, even maximal, of any single afferent nerve evokes contraction of merely a fraction (2,3) of the total aggregate of the motor units of the muscle (1,2,3). This fraction, proper to the afferent, is individual to it in the sense that, though its units all pertain also to some one or another of the fellow excitatory afferents, the particular combination of motor units which it presents *in toto* is peculiar to the afferent in question. Under concurrent stimulation of any two afferents α and β, tetanic discharge evoked by either in motor units common to both precludes, for the corresponding tetanised muscle-fibres of these latter, if in complete tetanic contraction, any intensification for the time being of mechanical tension in response to the other afferent, in short, "occlusion" ensues (4); though, as was shown, "substitution by equivalence" may on opportunity occur interchanging α for β and *vice versa*, and thus help to maintain and prolong the existent mechanical tension. If the tetanic contraction due to α be not complete the advent of β may give responses which help to complete it. But in our experiments here reported the stimulation rates were always of frequency sufficient to give practically complete tetanic contraction. Hence, under concurrent strong stimulation of α and β the isometric contraction-tension (= number of discharging motor units), though greater then than under α alone or β alone, is less than the sum of that of the two reflexes taken singly. The amount of this deficit furnishes a measure of the extent to which α's group of discharging units overlaps with β's group of discharging units (4).

This countervailing overlap is however but one aspect of the overlap between the motor unit groups pertaining respectively to α and to β. With weak stimuli the concurrent effect of α and β shows often an excess of contraction-tension over the sum of the two weak reflexes taken singly (4). This argues that with weak stimuli each afferent, while fully activating a certain number of motor units, acts also on a further number subliminally, and that some at least of these subliminally influenced units

are common to α and to β, so that the concurrent subliminal excitations sum to supraliminal effect. Since the motor units which, under concurrent weak stimulation of α and β, augment the combined effect by central summation of subliminal excitation, must be common to α and to β, and since, as shown, α and β have common units which under strong stimulation occlude, those units which under weak stimuli *summate* may well be those same ones which under strong stimuli *occlude*. Hence it has remained an open question whether under maximal stimulation of an afferent the fractional group of motor units on which it acts presents in addition to units which it discharges other further units on which it exerts merely subliminal effect. In short, evidence is desired as to whether the fringe of subliminal influence, which is traceable as a feature under weaker stimulation of the afferent, has its counterpart also under maximal stimulation of the afferent.

Under conditions and with technique similar to those of the work earlier reported (3, 4), further experiments now bring evidence of a fringe of subliminal effect centrally accompanying the reflex discharge evoked by strong just as well as by weak stimulation of the afferent nerve. Thus, in *m. tensor fasciæ femoris*, a hip-flexor, the ipsilateral internal saphenous nerve under strong faradic stimulation (50 per sec.) responded by a strong tetanic reflex contraction (Fig. 1, *A*, *s.* 13). A single break-shock stimulus to ipsilateral musculocutaneous afferent nerve evoked, when alone, a twitch-like peaked contraction-response declining fully to resting tension within $\frac{1}{5}$ sec. (Fig. 1, *A*, *mc* [control]). When however the break-shock stimulus to musculocutaneous was delivered during the plateau of the reflex tetanus (evoked through internal saphenous nerve) there resulted an immediate abrupt increment of contraction, and this increment, instead of subsiding within $\frac{1}{5}$ sec., persisted without much diminution during the remainder ($\frac{4}{5}$ sec.) of the continuance of the stimulation of the internal saphenous nerve (Fig. 1, *A*). It will be seen that of the motor units excited to discharge by the break-shock stimulus to the musculocutaneous afferent some were occluded owing to preoccupation effected by the precurrent tetanic stimulation of internal saphenous. This is evident from the deficit in tension of the break-shock response in Fig. 1, *A*, as compared with Fig. 1, *A*, *mc* [control]. The remainder of the units, *i.e.* those which were not being discharged by the stimulation of internal saphenous, were, however, under influence from internal saphenous, for their discharge, initially evoked *via* musculocutaneous nerve, was then sustained, after the stimulation of musculocutaneous had passed, by the stimulation of internal saphenous. The stimulation of internal saphenous,

while it continued, maintained the discharge of the incremental units initially evoked by musculocutaneous. Here the incremental motor units

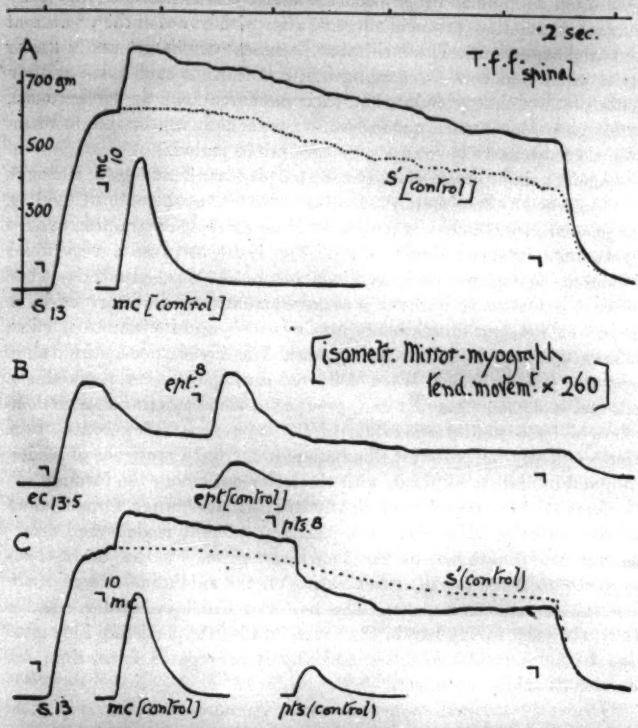

Fig. 1. *A*. Myograph; *m. tensor fasciæ femoris*, reflex from *s*, int. saph., 13 cm. coreless coil, 2 volts in primary, 50 per sec. frequency; *s'* (control), without addition of single shock reflex of musculocutan. at 10 cm. coreless coil. *B*. *ec*, ext. cutaneous, 13·5 cm. coreless coil at 50 per sec.; *ept*, ext. plantar nerve, 8 cm. of coil. *C*. *s* as in *A*, and at 13 cm. coil; *mc*, as before; *pts*, both plantar nerves, 8 cm. at 8 cm. coreless coil. Ordinates of tension. Time in fifths sec. Tracings from photogr. records; mirror myograph (9).

were contained within α's subliminal fringe until β influenced them and made α supraliminal in regard to them.

A counterpart to this was met in regard to tetanic reflexes of the same

two nerves, int. saphenous and peroneal, both of them excited by strong stimuli and in overlapping sequence. In this case the persistence of a facilitated subliminal fringe occurred under maintenance of the post-current stimulation (peroneal afferent) after withdrawal of the precurrent (internal saphenous). The facilitated persistent discharge was in motor units which had been discharging under precurrent saphenous and, on withdrawal of that stimulus, had their discharge kept up by peroneal, although peroneal when not forerun by saphenous was unable to maintain their discharge beyond a very brief initial period.

Again, a single break-shock to external plantar afferent *ept* 8, although evoking when by itself merely a small low twitch-like contraction (Fig. 1, *B*, *ept* [control]), was found, when added to an existent contraction evoked by external cutaneous nerve, *ec* 13·5 (Fig. 1, *B*), to cause a very much enhanced, though not prolonged, contraction-response. Posterior tibial afferent is known to harbour a certain amount of inhibitory effect in respect of this same muscle *tensor fasciæ femoris*, and its inhibitory effect follows upon an initial excitatory one. The break-shock stimulus of posterior tibial afferent, when delivered during the tension-plateau of internal saphenous tetanic reflex, produced a small twitch-like increment followed by a partial relaxation of the saphenous reflex contraction. Here evidently the posterior tibial afferent exerted a sequence of excitation and inhibition admixed, with the latter outlasting the former.

The inhibition is seen more clearly still (Fig. 1, *C*) when a break-shock to the posterior tibial (*i.e.* both plantars) afferent is delivered (*pts* 8) during the persistence of the increment given by the break-shock musculocutaneous (*mc* 10) reflex acting on the subliminal fringe of the internal saphenous response. The posterior tibial excitatory effect is then, although falling late in the course of the still persisting increment due to musculocutaneous, occluded by it as regards excitation, but unfolds its inhibitory effect.

Evidently internal saphenous nerve stimulates subliminally some motor units which it does not, acting by itself alone, cause to discharge. A discharge in these units once set up however by musculocutaneous afferent, in whose discharging fraction they are included, is then carried on by excitation from internal saphenous nerve, previously subliminal in regard to them. This does not ensue with posterior tibial afferent in Fig. 1, *B*, and its absence suggests that here the non-appearance is associated with the inhibition which is obviously present. The horizontal plateau observed in the internal saphenous reflex after posterior tibial inhibition (Fig. 1, *C*) argues that the motor units inhibited are just those

which had been under barely supraliminal excitation, so that a brief inhibition sufficed to reduce their central stimulation to subliminal then and for a considerable after-period.

The observations instance therefore certain afferent nerves which in regard to the flexor motoneurone-aggregate of the limb have a potential field of excitation wider than their field of motoneurones which they actually discharge. Such observations, in harmony with others, signify that the reflex discharge is determined by a grading of the central stimulation brought to bear upon the individual motoneurones severally of the motor unit aggregate. A factor in that grading for each individual motor unit would, from the above observations, be the richness of convergence of excited afferent terminals upon that particular unit. The greater the number of terminals which are in active play upon the motor unit the greater *cæteris paribus* the "central stimulation" at work upon it. A threshold stimulus to the afferent nerve causes motor discharge therefore in those motor units upon which the afferent terminals, derived from the few individual low-threshold nerve fibres in the afferent nerve, converge and overlap in richest number, *i.e.* in those motor units which receive in greatest density the excited terminals. With the same nerve a stronger stimulus brings into play additional afferent nerve fibres and therefore additional excited central terminals, some of them bearing on those same central units previously excited and some on central units in regard to which previously the convergence was too sparse to bring about actually their discharge, *i.e.* where the excitatory influence (the central stimulation) was previously subliminal. Some of these latter are by the stronger stimulus thus caused to discharge, and these thus pass over from the subliminally stimulated to the discharging (*i.e.* supraliminally stimulated) portion of the central group. The observations now adduced show that even when the stimulation of the afferent nerve is maximal, *i.e.* excites all the afferent fibres of the nerve, there results besides the discharge (*i.e.* supraliminal stimulation) of the maximal number of motoneurones for which that particular afferent is then competent, an accessory fringe of subliminal stimulation of other motoneurones. This latter group is demonstrable in various ways, *e.g.* by interaction yielding summation, facilitation, etc.; perhaps it corresponds with the field of enhancement by which strychnine augments the fractional group (3).

The above observations distinguish therefore under "central stimulation" of the motor units three grades of stimulation: (1) maximal, abundantly above threshold, (2) just supraliminal, and (3) just subliminal. There is however much that amplifies this glimpse, and indicates that an

excitatory afferent nerve playing upon its motoneurones (which themselves react on the all-or-none principle) of the flexion reflex does so in such a way that the "central stimulation" at different individual units presents between its extreme degrees at different units a long series of intermediate grades affecting other units, so that the stimulations at individual units, when the whole group of units is kept in view, present very many different degrees of stimulation both above and below threshold. The great variety in the duration of after-discharge in the different motor units composing any reflexly excited group of motoneurones is an expression of this.

An interest attaching to demonstration of the existence of a fringe of concomitant subliminal stimulation as part of the central activation field of an excitatory afferent nerve, whether weakly, moderately or maximally stimulated, lies in the probability that by such a subliminal fringe is afforded the opportunity for "enhancement," "reinforcement," "facilitation," etc., and other interactions of like kind between "centres," *e.g.* higher and lower, an interaction which is frequent and fundamental as a factor in regulation and in coordination.

Conclusions.

In the spinal flexion-reflex (hind limb, cat) the interaction between allied excitatory afferents reveals existence centrally of a fringe of subliminal excitatory effect with strong and maximal, as well as with weak, stimulation of individual afferent nerves. A fringe when once lifted above threshold may in some cases be then maintained there by a stimulus which previously was subliminal for it. Weak inhibition readily antagonises this maintenance.

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NOTE ON THE ELECTRIC RESPONSE OF NERVE TO TWO STIMULI.

By YNGVE ZOTTERMAN[1].

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In a previous paper by Gerard, Hill and Zotterman (1) on the effect of frequency of stimulation on the heat-production of frog's nerve, the return of the power of liberating energy after a previous impulse was given as a function of the interval between the stimulus and its predecessor. The "total action current" also at various frequencies of stimulation was recorded with a sensitive Downing galvanometer, and it was shown that this bears a relation to the frequency of stimulation which is very similar to that shown by heat liberation.

It was evident from these experiments that the power of liberating energy, as well as that of developing action potential, did not return completely to its full value in less than 50 σ after stimulation. From the work of Lucas and Adrian (2) it is well known that the return of excitability in a frog's sciatic nerve is complete in about 15 σ, and it has been shown that the return of the power of propagating the disturbance coincides with the return of excitability. The curve of recovery of excitability, however, was constructed by measuring the least interval for minimal muscular summation, and is therefore an expression of the return of excitability of the quickest motor fibres only of the nerve, while the experiments of Gerard, Hill and Zotterman referred to all the fibres. It was obviously desirable to confirm the results obtained on nerve stimulated at various frequencies, by observations on the total electric response to two stimuli only, with various intervals between them. This is readily done owing to the extreme sensitivity available in the high resistance Downing galvanometer employed.

Method. The nerve was placed in a Lucas chamber. The stimulating electrode was placed at the spinal end, non-polarisable electrodes of the zinc-zinc-chloride-gelatin-Ringer type at the distal end; one of these was

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on the crushed end of the nerve, the other about 20 mm. above the injury. In order to avoid current spread from stimulus to galvanometer the distance between the stimulated point and the effective off-lead was kept at about 30 mm. After killing the nerve between the point of stimulation and the off-lead, controls showed that the exciting current did not reach the galvanometer. Two break shocks, at different intervals, from two induction coils, were sent in by means of a Lucas spring contact-breaker. The first shock secured a maximal response, the second shock was taken ten times as strong or more if required. The monophasic electric response was recorded by a sensitive high resistance galvanometer constructed by Downing(3). This galvanometer with a moving magnet system of extreme lightness and a resistance of 14,800 ohms, gave a deflection up to 80 mm. in less than half a second for a single maximal shock. The injury current was balanced off and the maximal deflection for a single break shock was recorded. Two break shocks at different intervals were then sent into the nerve and the summed deflection was read ballistically on the scale. Subtracting from this the reading for a single maximal shock the effect of the second shock was obtained. Fifteen reliable experiments were performed on 15 different nerves, with intervals between the shocks rising from 1·4 σ to 30 σ and returning. Greater intervals were obtained by using a Lucas revolving contact-breaker in place of the spring contact-breaker.

Results. The effect of the second shock, obtained as above by subtraction, was expressed as a fraction of the effect of a single shock. The curve in Fig. 1 is drawn through points representing the average of observations on 15 different nerves[1]. This curve is in fairly good agreement with the one which can be constructed from the relation between frequency of stimulation and total action current(1). The action current does not reach its full value within 50 σ. For comparison the return of the power of liberating heat(1) is plotted in the same figure. It is obviously not as rapid in its earlier stages as the return of the action current. The latter, however, is measured ballistically as an amount of electricity, and the two curves are not directly comparable with each other since amount of electricity is proportional to CV, C being capacity and V potential, while energy is proportional to CV^2. Thus to compare the energy of a single maximal electric response with the energy of a second electric response we should consider the *squares* of the deflections obtained

[1] Three readings were made at each of six standard intervals, appropriately spaced, viz. 1·4, 2·8, 4·3, 9, 15 and 28 σ. The means were taken and averaged for all the nerves. The curve "return of action current," Fig. 1, is interpolated through the six points so obtained.

ballistically on the galvanometer scale. This has been done in Fig. 2. The curve so obtained is very similar to that for the return of heat production.

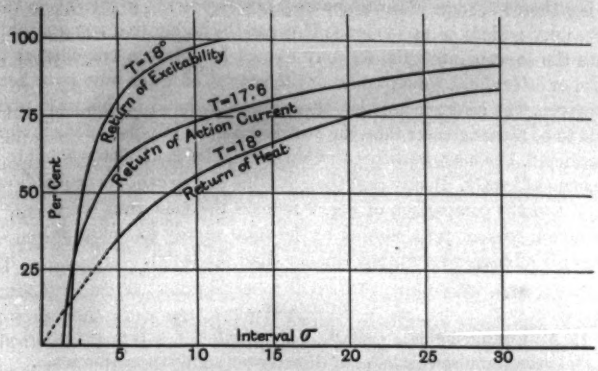

Fig. 1. Return of heat, action current and excitability in the response to a second shock, as the interval is increased.

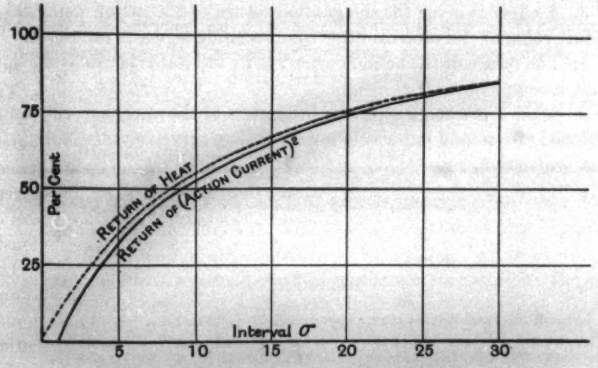

Fig. 2. Return of heat and of (action current)^2 in the response to a second shock, as the interval is increased.

One objection may be raised to the comparison in Fig. 2. The return of heat liberation in nerve is necessarily determined in such a way that the readings for maximal stimulation for 10 seconds at different frequencies

are divided by the frequency and by the duration of stimulus to give the heat per impulse. At frequencies above 200 per sec., that is with an interval of less than 5 σ, some of the fibres may not respond to every shock, their refractory periods being too long. Thus, at the higher frequencies, that is with the shorter intervals, we may expect to obtain a response at the third or later shock which would not appear at all when only two shocks are given. The heat per impulse, therefore, calculated in this way, would tend to be rather greater than the heat in a second impulse at the interval considered. The heat, however, in a single impulse is much too small to be measured directly. In any case the error due to this cause is probably not large, and the comparison of Fig. 2 favours the view that the energy in the action current runs parallel to the total energy in the impulse. The former is of course very much smaller than the latter.

SUMMARY.

1. An arrangement is described by which the action potential of a single nerve impulse can be read directly on a galvanometer scale.

2. Subtracting the reading for a single maximal shock from the reading for two shocks at any desired interval, the magnitude of the electric response to the second stimulus can be determined.

3. A curve is given for the gradual return of the action potential to its full value as the interval after the preceding shock is increased.

4. The return of the action current to its full value is relatively slow, being incomplete in 50 σ.

5. After a preceding stimulus the return of the energy in the action potential of a second impulse follows a similar curve to the return of the heat production.

I wish to express my thanks to Prof. A. V. Hill for much helpful advice in this work.

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THE OXYGEN CONSUMPTION OF THE FROG'S HEART. I.

By A. J. CLARK and A. C. WHITE.

(*From the Department of Pharmacology, University of Edinburgh.*)

Previous workers have studied the oxygen consumption of the frog's heart by means of two methods. One method has been the estimation of the amount of oxygen removed from suspensions of red blood corpuscles in contact with the heart (Weizsäcker(1, 2, 3), Gottschalk(4), and Lüscher(5, 6, 7) and Scheinfinkel(8)). A second method has been to measure the oxygen removed from air in contact with fluid circulated through the heart (Bodenheimer(9), Bohnenkamp and Friedmann(10), and Fukuda and Naito(11)). The present writers used both methods.

Methods.

Rana temporaria were usually employed, but sometimes *R. esculenta*. We found in agreement with Weizsäcker(1) that there was no significant difference in the oxygen use of the hearts of the two species. The Ringer's fluid employed had the following percentage composition: NaCl, 0·65; KCl, 0·015; $CaCl_2$, anhy., 0·012; sodium phosphate, 0·05; *p*H, 7·5. All experiments were done at room temperature which always lay between 12° and 15° C.

Three different methods were used.

Method 1. A Barcroft apparatus with bulbs of a special shape was used (Fig. 1). The volume of each bulb was about 12 c.c. The manometer of the apparatus was filled with kerosene (B.P. 220–240° C.). The frog's heart was fitted with a venous cannula (*V*), holding 1 c.c., and with an aortic cannula which returned the fluid to the venous reservoir. The drip provided for the oxygenation of the fluid. The heart was either allowed to beat with a normal rhythm or else a Stannius ligature was applied and the heart was driven with break induction shocks through the electrodes (*E*). A dummy cannula filled with Ringer's fluid was put in the control bulb. In some experiments an apparatus with larger bulbs (50 c.c.) was used and in this case a venous cannula was used which held 5 c.c.

The special advantages of this apparatus are that the oxygen consumption can be recorded continuously, and that the same fluid can be

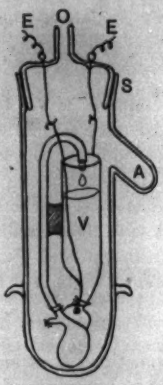

Fig. 1.

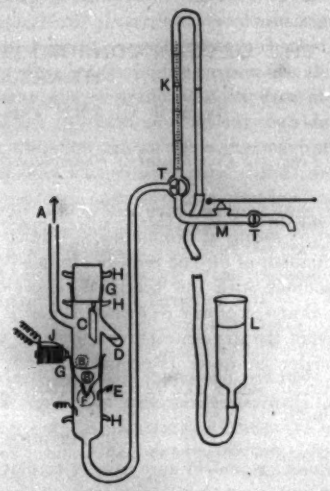

Fig. 2.

Fig. 1. Modified bulb of Barcroft apparatus (Method 1). *O*, connection to three-way tap of Barcroft apparatus of usual pattern. *E*, *E*, electrodes. These are hooked on to electrodes which run down to the auricle. The termination of one electrode is shown. *S*, ground glass joint. *A*, recess to hold 0·5 c.c. 10 p.c. caustic soda. After this fluid has been put in, the recess is plugged lightly with a roll of filter paper. The orifice of the recess is greased to prevent creeping of the caustic solution. *V*, venous reservoir. The aortic cannula and the electrodes are held in place by telegraphic cement and silk ligatures. The stopper is held on to the bulb by elastic bands passing over the hooks at the bottom.

Fig. 2. Apparatus used for Method 2. The cannula, to which the frog's ventricle (*F*) is attached, fits into a second cannula filled with boiled saline and is closed by a stopper. The joints (*G*, *G*) are ground glass and the three pieces are held together by elastic bands passing over the hooks (*H*, *H*, *H*). The side tube *D* contains caustic soda. The Ringer's fluid in the cannula (0·5 c.c.) is aerated by the glass ball (*B*, *B*) which is filled with iron filings, and which is made to jump up and down by currents passing through the electromagnet (*J*). The air in the cannula is stirred by the flapper *C*, which is an iron strip coated with collodion.

The upper cannula is connected by *A* to the three-way tap of a Barcroft manometer. A dummy tube is fixed to the other tap of the Barcroft manometer. The lower cannula is connected through the three-way tap (*T*) to the graduated tube (*K*), or to a membrane manometer (*M*). The diastolic pressure is regulated by altering the level of the vessel (*L*).

left in the heart for hours. Moreover, the heart receives an adequate oxygen supply when perfused with Ringer's fluid without the addition of red blood corpuscles. The apparatus was very convenient for the study of the development of the hypodynamic condition due to prolonged perfusion with Ringer's fluid. On the other hand, the apparatus was unsuitable for the measurement of the relation between oxygen use and work done because the initial filling of the ventricle could not be measured. A rough estimate of the mechanical activity of the heart was obtained by counting the drops of fluid circulated per minute.

Method 2. The apparatus shown in Fig. 2 was devised to permit the simultaneous measurement of the oxygen consumption and the mechanical response of hearts containing Ringer's solution. The ventricle (*F*) alone was used, and was driven at a constant rate by break induction shocks passing through the electrodes (*E*). About 0·5 c.c. fluid was put into the cannula. The fluid was oxygenated and stirred by the movements of a glass ball (*B*) filled with iron filings, which was made to jump up and down by means of an electromagnet outside the glass chamber, through which a current was passed about twenty times a minute. The air above the fluid was also stirred by the movements of a strip of iron (*C*) hanging from a loop of silver wire. This arrangement was designed by Mr N. E. Condon, assistant in this department.

The volume changes were measured at the air-water level in the graduated tube (*K*) and the initial tension was adjusted by the moveable water level (*L*). The systolic tension produced on isometric contraction was measured periodically by introducing the small membrane manometer (*M*) by means of the three-way tap (*T*).

The oxygen consumption was measured on a Barcroft manometer. Before taking a reading it was necessary to stop the stimulus, cut out the membrane manometer (*M*) and adjust the air-water level in the tube (*K*) to a fixed point by moving the water level (*L*). The tap (*T*) was then closed. When it was desired to measure the oxygen consumption of a heart at rest the heart was irrigated by emptying and filling it by the raising and lowering of the water level (*L*) about twelve times a minute.

The disadvantage of this method was that the stirring arrangement introduced oxygen and removed carbon dioxide at a rate only just adequate for the heart's needs.

Method 3. We also employed the method used by Weizsäcker(2) in which the ventricle was filled with a suspension of ox red blood corpuscles in frog's Ringer; this fluid was covered with liquid paraffin, and the amount of oxygen remaining in the blood corpuscles after a given period

was estimated by Haldane's blood gas analysis apparatus. The volume and tension changes in the ventricle were measured in the same manner as in Method 2. One c.c. of fluid was used, it was left in for 30 minutes and as much as possible (usually 0·8 c.c.) was removed for analysis. In this method the irrigation of the heart is imperfect, but the large oxygen content and the powerful buffer action of the corpuscles keep the heart in good condition if the fluid is changed every 30 minutes.

Method 1 was used with 70 hearts, Method 2 with 20 hearts and Method 3 with 11 hearts. There was a general agreement in the results obtained by the three methods. Methods 1 and 2 gave a continuous record of the oxygen consumption of the heart.

Fig. 6 shows a typical record obtained with Method 1. This figure shows that a change in the rate of oxygen consumption was recorded in the first 5 minutes after a change of frequency, and therefore this apparatus was remarkably free from "lag." Fig. 7 shows a record obtained by Method 2. With this method it was found that changes in oxygen consumption were only recorded after a delay of about 15 minutes. This difference in amount of delay in the two methods is an indication of the difference in the efficiency of the aeration of the fluid. When the fluid in the heart was changed in either the first or second methods, we allowed 15 minutes for equilibrium, and after this it was usually found necessary to take readings for 45 minutes in order to establish the rate of oxygen consumption with certainty.

The influence of initial filling.

All observers agree that the oxygen consumption of the heart is profoundly influenced by the initial filling. Starling and Visscher(13), working with the heart-lung of the dog, found that the amount of oxygen consumed in a contraction was determined by the diastolic volume of the heart, and was not influenced by the resistance against which the heart contracted.

Lüscher(7), who used the frog's heart, admitted the profound influence of initial filling, but thought that the resistance also influenced the contraction. Starling and Visscher(13) showed, however, that his results really accorded best with the view that they put forward.

Bohnenkamp and Ernst(14) concluded from a study of the heat production of the heart that an equal amount of energy was released with each contraction and that variation in such mechanical conditions as the initial filling did not alter the quantity of energy set free but only altered

the proportion of the free energy that was converted into work. This conclusion is opposed to the results obtained by all other workers. Fischer(15) showed that Bohnenkamp's method had a large experimental error, and with an improved method he showed that an increase in the initial tension caused an increase both in the work done and also in the heat produced.

Fig. 3 shows a typical result obtained by us using Method 3. This

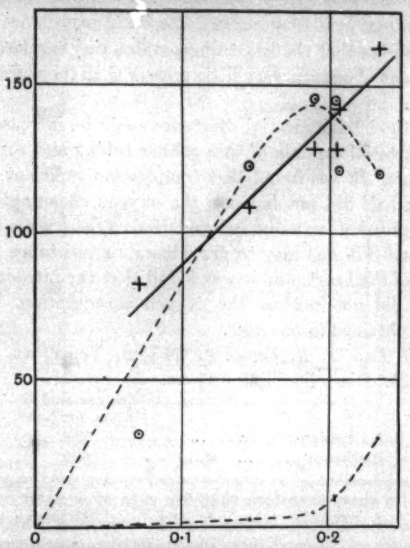

Fig. 3. The relation between diastolic volume, oxygen consumption and systolic pressure (Method 3). Abscissa—diastolic volume in c.c. Continuous line and crosses oxygen consumption in c.mm. per hour. Broken line and dots, diastolic pressure in cm. water. Broken line and circles, added systolic pressure in cm. water ×2. (Method 3, Exp. 4. Ventricle weight, 130 mg. Rate constant at 24 per minute.)

figure indicates that the empty heart uses about 25 p.c. as much oxygen as the full heart, but that if this "resting" metabolism is deducted there is a simple linear relation between the initial filling and the oxygen used.

Similar results were obtained with Method 2, and Table I shows figures obtained with Method 1.

TABLE I. Frog's heart perfused with Ringer's fluid (Method 1).

Exp.	Heart weight in mg.	Rate	Oxygen consumption in c.mm. per hour	
			Full	Empty
59	82	16	118	36
—	—	0	34	31
60	79	16	50	10
—	—	0	10	10
64	81	26	72	24
33	28	32	66	41
—	—	0	—	32

Table I shows that the heart when contracting regularly, but empty, uses an amount of oxygen very little greater than its resting metabolism when empty.

With Method 1 the arterial resistance could be increased by making part of the arterial cannula of thin rubber tubing and compressing this with a ligature. It was found that compression sufficient to reduce the outflow to a half did not increase the oxygen consumption, and that further compression actually reduced the oxygen consumption. With Methods 2 and 3 it was easy to provide an air resistance to oppose the contraction of the heart, and it was found that the introduction of such a resistance did not increase the oxygen consumption. The following figures were obtained in one case.

Method 3, *Exp.* 3. *R. Temp.* ♂. 29 grm. Ventr. wt, 67 mg. Rate constant at 24. Diastolic filling 0·11 c.c.

	Oxygen used in c.mm. per hour
No resistance	62·8
Resistance	60·0

Our results show therefore that the rate of oxygen consumption is controlled by the diastolic volume of the ventricle, which again is determined by the pressure to which the ventricle is subject during diastole. Increase in the resistance against which the ventricle contracts does not, however, increase the oxygen consumption, and when this resistance is increased sufficiently to interfere seriously with the emptying of the ventricle, the oxygen consumption actually decreases. The probable reason for this decrease is that, as will be shown later, any accumulation of products of metabolism around the heart cells inhibits the oxygen uptake of the heart.

Our experiments also show that the empty hearts consume from 20 to 30 p.c. as much oxygen as a heart in full activity. A similar amount of oxygen is consumed by a resting heart either empty or full. The difference

between the oxygen consumption of an empty heart contracting regularly and that of an empty heart at rest was too small to be estimated with certainty by our methods. We believe that the difference is less than 10 p.c. of the extra oxygen consumption that is caused by contraction in a full heart.

Influence of rate.

Weizsäcker(3) found that when the frequency was varied, the oxygen consumption per beat remained nearly constant up to a frequency of 30 per minute, but that a further increase of frequency reduced the oxygen used per beat. Our results confirm this conclusion. Fig. 4

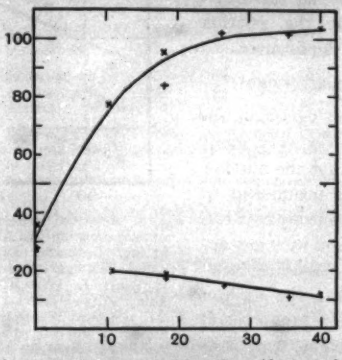

Fig. 4. Influence of frequency on oxygen consumption. Abscissa—frequency in beats per minute. Upper line—oxygen consumption in c.mm. per hour. Lower line—drops per ten beats. (Method 1, Exp. 5: heart weight, 66 mg., +; Exp. 23: heart weight, 97 mg., ×.)

shows typical results obtained with Method 1. This figure also shows that the oxygen consumption of a heart at rest is about 30 p.c. of that of a heart contracting at a rate of 30 per minute.

Individual variation in oxygen consumption.

An examination of the figures given by any of the workers who have measured the oxygen consumption of the frog's heart shows that there is a great variation in the rate of oxygen consumption in different hearts of similar weight. We found that this variation was very marked. The figures given in Table I illustrate this point, for in two hearts of similar weight (Exps. 59 and 60) the oxygen consumption was respectively 50 and 118 c.mm. per hour. This variation was equally marked in the results

obtained with all three methods. Table II shows the average figures obtained with hearts of varying size with the three methods used. It will be seen that on the average the larger hearts had a larger initial volume, a more powerful mechanical response and a higher oxygen consumption. These averages are shown in Fig. 5. The results from Methods 1 and 2 suggest that the oxygen use does not vary directly as the heart weight, but that the relation approximates to the formula

$$\text{oxygen use} \propto (\text{heart weight})^{\frac{3}{2}}.$$

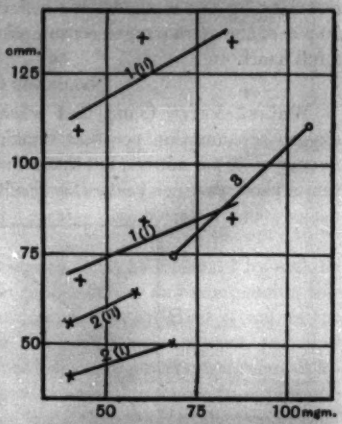

Fig. 5. Relation between heart weight and oxygen consumption. Abscissa. Heart weight in mg. Ordinate, oxygen use per hour in c.mm. The figure shows the averages given in Table II. 1 (i) = Method 1 and Ringer's fluid; 1 (ii) = Method 1 and diluted serum. 2 (i) = Method 2 and Ringer's fluid; 2 (ii) = Method 2 and diluted serum. 3 = Method 3, red blood corpuscles suspended in Ringer's fluid.

The individual variation observed was however very great and we consider that the number of observations is insufficient to establish the relationship between size and metabolism with any accuracy. The figures suffice, however, to show that the oxygen use is not directly proportional to the heart weight, and that therefore it is inadvisable to express results as oxygen use per unit weight of heart.

Comparison of different methods.

Fig. 5 shows that in the case of Methods 1 and 2 the oxygen consumption with serum was considerably higher than the oxygen consumption with Ringer's fluid. The significance of this fact will be discussed in another paper, but the results with Method 3 can only be compared with the results obtained with Methods 1 and 2 in the presence of serum.

Experiments with the isometric auricle showed that the frog's auricle produces an initial pressure of 10–20 cm. of water. This therefore was the approximate initial pressure with Method 1, and the figures for Methods 2 and 3 were taken from experiments in which similar diastolic pressures were employed. The initial filling was similar in all methods, as is shown in Table II. The mechanical conditions in the three methods are therefore

TABLE II. Average figures for oxygen use and mechanical activity, obtained with different methods.

Method	Rate	Perfusion fluid	No. of exps.	Average weight of hearts in mg.	Average oxygen use in c.mm. per hour	Average initial volume in c.mm.	Average added systolic pressure in cm. water
1	24	Ringer	6	42	66	(80)	—
			10	60·4	84·6	(90)	—
			6	84	85	(120)	—
		Serum diluted with Ringer	6	42	109	(80)	—
			10	60·4	135	(90)	—
			6	84	133	(120)	—
2	18	Ringer	12	40	41	87	61
			6	68	50	112	71
		Serum diluted with Ringer	4	40	56	100	65
			2	57	65	100	80
3	24	Ox red blood corpuscles in Ringer	5	68	75	130	54
			4	106	111	200	60

(1) With Method 1 only those experiments are averaged in which the oxygen consumption was determined both with Ringer and with serum. The initial volume in Method 1 was estimated from the output per beat.

(2) The experiments with Methods 2 and 3 were conducted with initial pressures of 10 to 20 cm. of water.

(3) In all cases the figures were taken after hearts had been isolated for more than an hour.

comparable. The results with Method 2 should, however, be multiplied by $\frac{24}{18}$ to compensate for the difference in rate.

A comparison of the results of Methods 1 and 2 shows that when the difference of rate is allowed for Method 1 gives results from 20 to 50 p.c. higher than does Method 2. Similarly the results with Method 1 are about 40 p.c. higher than the results with Method 3. In Method 1 the heart is functioning under conditions more nearly approaching the natural than in Methods 2 and 3. We believe that the irrigation of the heart cells and the aeration of the fluid were most efficient in Method 1, and that this is the reason why this method gave the highest values for oxygen consumption.

RESULTS OBTAINED BY OTHER WORKERS.

Table III shows averages extracted from the results obtained by other workers who used methods similar to our third method; namely, the introduction into the heart of a suspension of red blood corpuscles and the estimation of the oxygen removed after a certain interval. In all cases there was a great individual variation, even with hearts of the same weight. The averages were taken by us from those experiments in which

TABLE III.

Author	Number of experiments	Average weight of hearts in mg.	Average oxygen use in c.mm. per hour
Weizsäcker(1)	6	110	321
Weizsäcker(2)	10	100–200	142
Weizsäcker(3)	15	100–200	173
Gottschalk(4)	9	132	157
Lüscher(5)	3	122	271
Scheinfinkel(6)	11	—	382

the rate was between 20 and 30 and the initial pressure was 10 to 15 cm. of water. Under these conditions we obtained with Method 3 an oxygen use of about 100 c.mm. per hour for a heart of 100 mg. Weizsäcker's first figures and the figures of Lüscher and Scheinfinkel are far greater than our figures; but the results in Weizsäcker's second and third papers and Gottschalk's results are about 60 p.c. higher than ours, and this difference can be accounted for by the difference in temperature which in our experiments was about 13° C. and in their experiments about 18° C.

Fukuda and Naito(11) have measured the oxygen consumption of frog's hearts, using a Straub cannula filled with Ringer. With an initial pressure of 6 cm. of water they obtained figures of 52–65 c.mm. oxygen per hour per 100 mg. of heart. This method is comparable to our Method 2, and extrapolation of our figures with this method gives an average value of 70 c.mm. oxygen per hour per 100 mg.

Fischer(15) measured the heat production of the ventricle of *R. temp.* of medium size. The ventricle was filled with Ringer's fluid. He found that with an initial pressure of 10 grm./cm. the heat production was equivalent to 2·1 grm./cm. and the work done to 1·6 grm./cm. The total energy set free was therefore 3·7 grm./cm. If we assume that there is a recovery process in cardiac muscle similar to that of skeletal muscle, then the total energy exchange per contraction will become $3{\cdot}7 \times 2{\cdot}1 = 7{\cdot}8$ grm./cm. This corresponds to an oxygen consumption of nearly 0·04 c.mm.

Table II shows that the average oxygen consumption of ventricles perfused with Ringer's fluid (Method 2) and beating 18 times a minute was 40 to 50 c.mm. oxygen per hour. This corresponds to an oxygen consumption per beat of 0·037 to 0·046 c.mm.

RESTING METABOLISM.

Weizsäcker(3) measured the resting metabolism of the frog's heart, and his average figure for hearts with an average weight of about 150 mg. was 18 c.mm. oxygen per heart per hour. He concluded that the resting

metabolism was about 4 to 8 p.c. of the metabolism during activity. Fukuda and Naito(11) found that the resting metabolism was 13 c.mm. O_2 for 100 mg. frog heart per hour. Weizsäcker's figures for metabolism during activity were, as we have already mentioned, about double those obtained by us.

We measured the basal metabolism by Method 1 in 20 hearts. The figures varied from 12 to 80 c.mm. oxygen per hour with an average figure of 30 c.mm. oxygen per hour for an average heart weight of 65 mg. With Method 2 we obtained a figure of 12 c.mm. oxygen per hour for a heart of 40 mg., and with Method 3 figures of 18 and 14 c.mm. oxygen per hour for hearts of 130 and 103 mg. respectively.

Our figures for resting metabolism with Method 1 represent a resting oxygen use of about 35 p.c. of the oxygen use in moderate activity, whereas with Methods 2 and 3 our figures are nearer those obtained by other workers.

We believe that the results obtained with Method 1 are nearest the truth, and that the resting metabolism is lower with the other methods because it is impossible to irrigate the heart surfaces thoroughly unless the heart contracts, and hence the heart's oxygen uptake is inhibited by the accumulation of products of metabolism, whereas in Method 1 respiration through the pericardial surface is not interfered with by arrest of the heart.

Oxygen debt.

Fig. 6 shows a continuous record of the oxygen consumption of a heart during work and during rest. The oxygen consumption at rest was first measured for 35 minutes, and then the heart was stimulated at 44 beats per minute. The oxygen consumption rose to 80 p.c. of the maximum in 10 minutes. This shows that the lag was relatively small. A stimulation rate of 44 per minute is considerably greater than the normal rate of the frog's heart *in situ*; higher rates could not be produced because *A–V* block occurred. When the stimulus was stopped (90 minutes) the oxygen consumption fell rapidly and reached a basal level in about 20 minutes. The total oxygen consumption in the first 20 minutes after cessation of the stimulus was 13 c.mm., whereas the oxygen consumption for 20 minutes at the basal rate of 17 c.mm. per hour would have been 5·7 c.mm. The excess oxygen consumption or oxygen debt was therefore 7 c.mm. A similar result was obtained when the stimulus was stopped at 275 minutes. The oxygen debt of 7 c.mm. equals the amount of oxygen consumed in about 3 minutes when the heart is contracting at 44 per minute. The figure of 7 c.mm. is a

maximum value for the oxygen debt, since no allowance has been made for instrumental lag, and the true value may be considerably smaller.

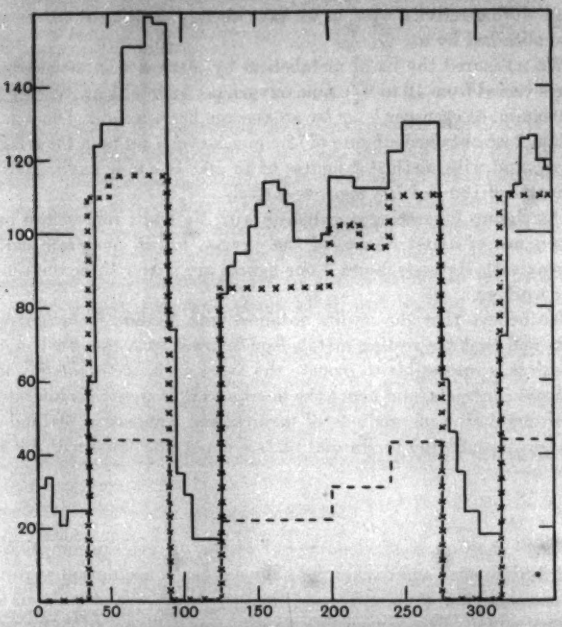

Fig. 6. Effect of changes of frequency on oxygen consumption. Abscissa—time in minutes. Continuous line—oxygen consumption in c.mm. per hour. Broken line—frequency in beats per minute. Crosses—drops per minute. (Method 1, Exp. 31: heart weight, 114 mg. Perfusion fluid defibrinated frog's blood.)

In this experiment the heart was driven at a rate that was considerably greater than its normal rhythm, but the aeration of the heart cells was good. The results show that under these conditions the oxygen debt is small.

Fig. 7 shows the behaviour of a heart perfused with Ringer's fluid when it was forced to do excessive work. This experiment was made with Method 2 in which the aeration of the fluid was only just adequate for the normal activity of the heart.

The diastolic volume of the heart was increased from 120 to 190 c.mm. by increasing the diastolic pressure from 20 to 35 cm. of water. The heart responded by an immediate increase in oxygen consumption, but it was unable to maintain this rate and the systolic pressure and oxygen consumption both fell. When the diastolic volume was reduced again to 120 c.mm. the oxygen consumption fell below the normal level for this volume. The contents of the heart were found to be acid (*p*H 6·8), and when replaced by fresh Ringer (*p*H 7·5), the oxygen consumption became normal again. The acidity of the fluid removed was due to CO_2 and not to lactic acid, for passage of a current of air through the fluid raised its *p*H to 7·5. Inadequate aeration of the fluid therefore caused an accumulation of carbon dioxide, and the increased acidity apparently inhibited the oxygen consumption of the heart.

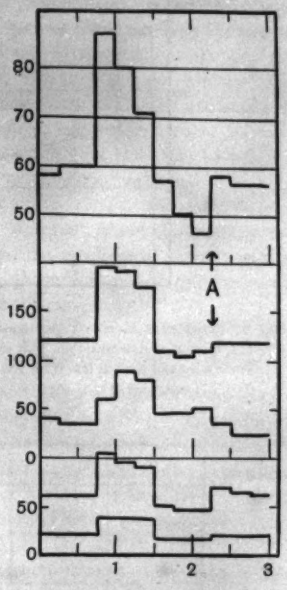

Fig. 7. Effect of accumulation of products of metabolism on oxygen consumption. Abscissa—Time in hours. The curves from below upwards represent (1) diastolic pressure and (2) systolic pressure; both in cm. of water; (3) systolic volume and (4) diastolic volume, both in c.mm.; (5) oxygen consumption in c.mm. per hour.

At 45 minutes the diastolic pressure was raised from 20 to 40 cm. and at 90 minutes it was reduced again to 20 cm. At 135 minutes (*A*) the Ringer's fluid was changed. The *p*H of the fluid removed was 6·8. (Method 2, Exp. 13: heart weight, 110 mg.)

Weizsäcker(2) also found that after either cyanide intoxication or asphyxiation of the heart there was never any supernormal oxygen consumption when fresh blood containing plenty of oxygen was introduced.

Influence of reaction on oxygen consumption.

Fig. 8 shows the effect of changes of reaction on the oxygen consumption and the mechanical response of the heart.

Accurate measurement of the exact influence of slight changes of reaction was impossible because only small volumes of fluid could be used and the production of CO_2 by the heart altered the reaction during the

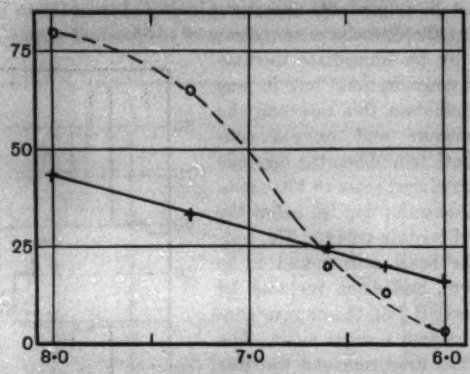

Fig. 8. Effect of acid on oxygen consumption and systolic pressure. Abscissa—*p*H. The crosses and continuous line show the oxygen consumption in c.mm. per hour. The circles and broken line show the added systolic pressure in cm. water. (Method 2, Exp. 17: heart weight, 31 mg.)

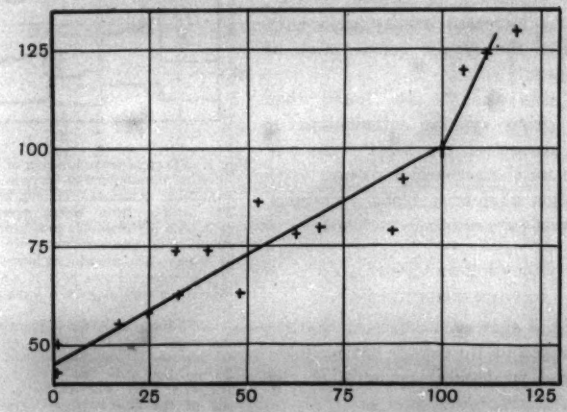

Fig. 9. The relation between the changes in oxygen consumption and the changes in mechanical response produced by alteration of reaction. The figures are taken from five experiments with Method 2. In all cases the figures have been reduced to percentages of the "normal" activity (*p*H 7·5). Abscissa—added systolic pressure. Ordinate—oxygen consumption.

course of an experiment. Perfusion of hearts with large volumes of fluid showed that the heart was arrested by a fluid of about *p*H 6·2.

Fig. 9 shows the relation between the oxygen consumption and mechanical response of the heart, with reactions varying from 6·2 to 8·0. This figure shows that an acidity sufficient to arrest the heart only reduces its oxygen consumption to about 50 p.c. of normal. Similar high residual oxygen consumptions were found when the heart was arrested by other ionic changes. If this residual oxygen consumption be subtracted then the reduction in oxygen consumption produced by acidity is proportional to the reduction in the mechanical response.

Increased acidity therefore reduces both the oxygen consumption and the mechanical response of the heart. Gray(17) found that acidity reduced both the oxygen consumption and the mechanical activity of the cilia of *Mytilus*, but that the oxygen consumption was only reduced 50 p.c. by an acidity sufficient to arrest all movement.

Anaerobic activity of the heart.

Weizsäcker(3) showed that a concentration of cyanide sufficient to reduce the oxygen consumption of the frog's heart by 80 p.c. only reduced the mechanical response by 30 p.c. We have confirmed this result with our methods. Other workers (Scheinfinkel(18) and Bachmann(19)) have shown also that the frog's heart if perfused with alkaline Ringer containing cyanide can continue to function fairly normally for many hours.

Weizsäcker(3) showed that the anaerobic activity of the heart in the presence of cyanide was associated with the excretion of a fixed acid into the perfusion fluid, whilst Andrus and Carter(20) found that the dog's heart was much less sensitive to lack of oxygen in alkaline than in neutral Ringer's fluid. It appears therefore that the frog's heart can function anaerobically, provided that it is perfused with a Ringer sufficiently alkaline to neutralise the acid produced.

On the other hand, our experiments show clearly that any accumulation of acid reduces both the mechanical response and the oxygen uptake of the heart.

Discussion.

The fact that the heart can continue to function fairly normally in the absence of oxygen, provided that accumulation of acid is prevented, shows that an anaerobic breakdown process can occur in the heart. Since accumulation of acid inhibits both the oxygen consumption and the

mechanical response, therefore acidity must inhibit the katabolic breakdown process.

Scheinfinkel(18) and Bachmann(19) both showed that if a perfused heart was stimulated too rapidly it passed into a state of apparent exhaustion from which it recovered spontaneously when the rate of stimulation was reduced, and that this recovery occurred equally well in the presence or absence of oxygen. They pointed out the contrast to the behaviour of skeletal muscle which when stimulated to exhaustion can only recover in the presence of oxygen.

The explanation appears to be that the heart is very sensitive to any accumulation of acid on its surface, and that apparent exhaustion is produced by accumulation of quite a small amount of acid, and that even an accumulation of CO_2 can produce sufficient acidity to cause partial inhibition of the heart.

Our experiments confirm those of Mines(22) and Andrus and Carter(20), who showed that the heart's activity was depressed by such a slight acidity as *p*H 6·7. On the other hand, the activity of skeletal muscle is only reduced about 50 p.c. by prolonged immersion in a fluid of *p*H 4·5 (Neugarten(21)). The heart is therefore about 100 times more sensitive than skeletal muscle to the effect of increase in hydrogen ions in the fluid surrounding its cells.

This explains the difficulty in forcing the heart to enter into oxygen debt, because if the circulation is impaired the accumulation of acid outside the heart cells depresses the catabolic breakdown process and checks further production of acid. Oxygen debt can only be produced if the heart is perfused with an alkaline oxygen-free Ringer's fluid. This hypothesis is supported by the results of Redfield and Medearis(23), who found that the lactic acid content of the turtle's ventricle at rest was 0·04 p.c. and that any increase above this figure caused a reduction in the mechanical response. They found however that stimulation of strips of ventricle in an atmosphere of nitrogen could cause the accumulation of about 0·175 p.c. of lactic acid. Gemmell(24) found in the same preparation a resting value of 0·026 and a fatigue value of 0·141 p.c.

Katz and Long(25) showed in the case of mammalian hearts that the increase in lactic acid on prolonged stimulation under anaerobic conditions was less than one-quarter of the increase in lactic acid in mammalian skeletal muscle under similar conditions.

There is therefore some accumulation of lactic acid in fatigue in the heart, and the difference between fatigue in cardiac and skeletal muscle appears to be one of degree rather than of kind.

SUMMARY.

1. The oxygen consumption of the frog's heart has been investigated by three methods, two of which are new.

2. The oxygen consumption can be divided into two portions: (*a*) the resting metabolism and (*b*) the extra oxygen consumption due to contraction. The resting metabolism of the heart is about 25 p.c. of the oxygen consumption during moderate activity.

3. The oxygen consumption due to contraction varies as the diastolic volume of the heart, but does not appear to be influenced by variations in the resistance against which the heart contracts.

4. An empty heart contracting regularly consumes very little more oxygen than an empty heart at rest.

5. Slight acidity (*p*H 6·7) decreases both the mechanical response and also the oxygen consumption of the heart.

6. Interference with the circulation through the heart diminishes both the mechanical response and also the oxygen consumption of the heart. This reduction can be explained by the accumulation of carbon dioxide and consequent increased acidity.

7. Our results can be explained on the assumption that the process of contraction in the heart is similar to that in skeletal muscle, but that slight acidity inhibits the initial anaerobic breakdown process in the heart, whereas skeletal muscle is relatively insensitive to the accumulation of acid.

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OXYGEN CONSUMPTION OF THE FROG'S HEART.
II. Effect of Lipoids, Ions and Narcotics.

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In the present investigation we used the same three methods for determining the oxygen consumption of the isolated frog's heart as are described in our previous paper (1).

A. The hypodynamic condition.

In all experiments in which the heart was perfused with Ringer's fluid for some hours a steady fall in oxygen consumption was observed. This was seen best when the heart was perfused with a considerable quantity of fluid, and a typical result is shown in Fig. 1. In this case

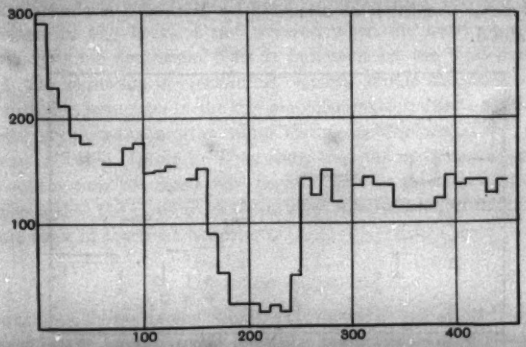

Fig. 1. Development of hypodynamic condition in frog's heart. Abscissa—time in minutes; ordinate—oxygen consumption in c.mm. per hour. Rate constant at 32, stimulus stopped between 160 and 240 minutes. (Method 1, exp. 22: heart weight, 138 mg. Heart perfused 5 c.c. Ringer's fluid; fluid not changed. The output remained constant at 100–120 drops per minute.)

5 c.c. of fluid were perfused through the heart. In other experiments where 1 c.c. or less of Ringer's fluid was perfused the oxygen consumption

fell at a slower rate, but there was a fall every time the heart was washed out.

This agrees with the observations of Vernon(2) who found that the carbon dioxide output of the tortoise heart diminished on prolonged perfusion with Ringer's fluid and that it was increased by addition of serum.

These results recalled the effects of prolonged perfusion on the mechanical response, described by one of the writers (3). The theory then advanced to account for the production of the hypodynamic condition was that perfusion washed out of the heart some substance, probably a soap, that was essential for the maintenance of the normal functions of the heart.

Our second method gave simultaneous records of the mechanical response and the oxygen consumption of the frog's ventricle perfused with a small amount (0·5 c.c.) of Ringer's fluid. The small amount of fluid was unfavourable to the production of the hypodynamic condition, but the fall in the oxygen consumption agreed fairly well with the results obtained by Method 1, and the mechanical response diminished in proportion to the fall in the oxygen consumption. The development of the hypodynamic condition as measured by Method 2 is shown in Fig. 2 and Table I.

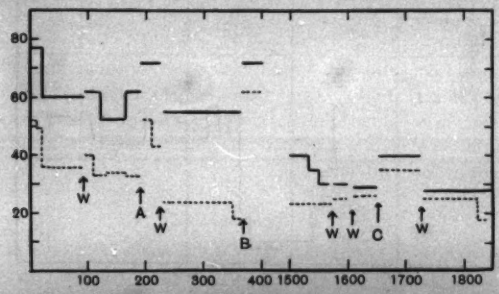

Fig. 2. Action of serum, etc. on hypodynamic heart. Abscissæ—time in minutes since commencement of experiment. Continuous lines, added systolic pressure in cm. water; dotted lines, oxygen consumption in c.mm. per hour. (Method 2, exp. 5: heart weight, 40 mg.) *W* = change of Ringer's fluid. *A* = frog's serum and Ringer's fluid equal parts introduced. *B* and *C* = alcoholic extract of ox serum (0·1 p.c.) introduced.

Table I shows that a considerable oxygen consumption persists when the mechanical response is almost abolished.

TABLE I.

Method 2, exp. 13: heart weight, 110 mg. Temperature, 13·5° C. Rate, 25. Heart contained 0·5 c.c. Ringer's fluid which was changed every few hours.

Time in hours since beginning	...	0	12	24	30	48
O_2 use in c.mm. per hour	... ...	75	40	50	50	23
Added systolic pressure in cm. water		85	34	32	32	2

Table II shows the averages for mechanical response and oxygen consumption, from seven experiments.

TABLE II.

Time since isolation in hours	0	1	2	3	6	24
(1) Total oxygen consumption as percentage of initial value	100	84	79	73	59	50
(2) Oxygen consumption due to contractile process (*i.e.* total oxygen consumption less resting oxygen consumption) as percentage of initial value	100	77	70	61	39	26
(3) Added tension	100	86	79	75	57	47

We have shown(1) that between 20 and 30 p.c. of the total oxygen consumption of the heart is due to resting metabolism, and this resting metabolism of the heart is not reduced when the heart passes into the hypodynamic condition. This is indicated by the final figures in Table I. The reduction in mechanical response should therefore be compared with the reduction in the oxygen consumption due to contraction (*i.e.* total oxygen consumption minus resting metabolism). These figures are given in Table II, line 2. They show that the oxygen consumption due to contraction is reduced more rapidly than the mechanical response. This means that the heart works somewhat more efficiently in the hypodynamic than in the fresh condition.

B. ACTION OF SERUM, ETC.

It has been shown by one of us(3) that the amplitude of contraction of the heart in the hypodynamic condition is stimulated in a remarkable manner by serum, alcoholic extracts of serum, saponified alcoholic extracts of serum, and even by sodium oleate. We repeated these experiments on hearts in which the isometric response was recorded and found that the effect on the isometric response was the same as that previously recorded on the isotonic response.

The typical response of the hypodynamic heart is given in Fig. 2, which shows that the introduction of serum or of alcoholic extract of

serum restores both the oxygen consumption and the mechanical response of the hypodynamic heart to the level observed in a fresh heart. Typical results obtained with Method 1 are shown in Table III. These figures show that frog's serum has a more powerful action than washed red blood corpuscles, and that alcoholic extract of serum does not lose any of its effect on saponification.

TABLE III.

Method 1, exp. 35: heart weight, 68 mg. Rate constant at 24.

Time in minutes ...	0–55	55–100	100–180	200–265
Fluid perfused	Ringer	Washed R.B.C. of frog + Ringer equal parts	Ringer	Frog serum, 1 part: Ringer, 2 parts
Drops per minute ...	92	92	87	90
Oxygen use c.mm. per 1 hr.	97	173	126	210

Exp. 45: heart weight, 56 mg. Rate constant at 24.

Time in minutes... ...	0–175	200–250	250–300	300–350	350–375
Fluid perfused	Ringer	Alcoholic extract of ox serum (0·01%) (=2% original serum)	Ringer	Saponified alcoholic extract of ox serum (0·01%) (=10% original serum)	Ringer
Drops per minute ...	75	75	60	60	60
Oxygen use c.mm. per 1 hr.	96	114	102	150	101

The influence of frequency on the oxygen consumption with blood and with Ringer's fluid is shown in Fig. 3. This shows that the resting metabolism is the same in the two cases. The blood, therefore, appears to increase the extra oxygen consumption associated with the contraction process, but not to affect the resting metabolism.

The nature of the active substance in serum.

We found that serum from the frog, ox or dog produced a similar stimulant action. A series of experiments was made with ox serum to discover the nature of the substance in serum that stimulated the oxygen consumption of the frog's heart. The tests were made roughly quantitative by estimating how much original serum a given quantity of any fraction represented. With the original ox serum the addition of 10 p.c. increased the oxygen consumption about 50 p.c. and 4 p.c. serum produced only about a 10 p.c. increase.

The serum was poured into ten times its volume of rectified spirit, filtered, evaporated to dryness in vacuo at 37° C., and the residue

extracted with absolute alcohol. This alcohol soluble fraction obtained weighed 0·5 p.c. of the original serum, and a concentration of 1 in 2000 (= 10 p.c. of the original serum) gave an increase in oxygen consumption of 69 p.c. Saponification of this extract by boiling with alcoholic potash did not diminish its activity (cf. Table III).

A number of experiments were made to try to purify the active principle, but it was found to be soluble both in petrol ether and in alcohol, and we were unable to concentrate it further.

Other experiments showed that lecithine and even sodium oleate gave an action similar to that produced by the saponified alcoholic extract. On the other hand, direct extraction of serum by shaking with petrol ether gave 0·1 p.c. of ether soluble fat which had little action on the heart, whereas the activity of the extracted serum was unaltered.

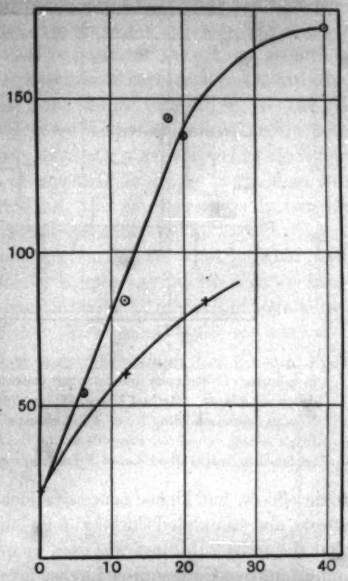

Fig. 3. Oxygen consumption at various frequencies with Ringer's fluid (lower curve) and defibrinated blood (upper curve). Abscissa—frequency, beats per minute; ordinate—oxygen use in c.mm. per hour. Upper curve—defibrinated frog's blood; lower curve—Ringer's fluid. (Method 1, exp. 27: heart weight, 87 mg.)

Prof. J. C. Drummond kindly gave us samples of non-saponifiable fractions of cod liver and shark liver oil, and these were tested on the heart with negative results. The evidence, therefore, indicates that the active substance in serum which augments the oxygen consumption is a soap. Experiments with pure sodium oleate showed that a concentration of 1 in 25,000 produced an increase in oxygen consumption of 70 p.c. (average of 7 experiments). Concentrations of less than 50,000 produced little effect.

Ox serum 10 p.c. produced an effect about equal to that produced by 1 in 25,000 sodium oleate, and therefore the action of serum would be accounted for by the presence of 0·04 p.c. soap in the serum.

The administration of small quantities of serum or of low concentrations of oleate to the heart produced an initial stimulation followed by a fall, as is shown in Fig. 4. Higher concentrations of oleate produced

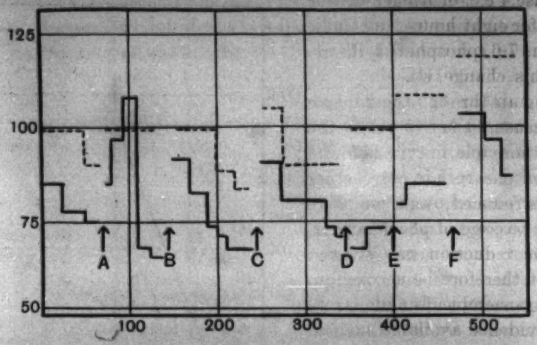

Fig. 4. Action of small quantities of frog's serum on oxygen consumption. Abscissa—time in minutes. Continuous lines, oxygen consumption in c.mm. per hour; broken lines, drops per minute. (Method 1, exp. 55: heart weight, 93 mg. Frequency constant at 30. Venous cannula holding 1 c.c. Fluid changed at arrows.) *A*, *B* and *C* = 1 c.c. of 1 p.c. frog's serum. *D* = 1 c.c. Ringer's fluid. *E* = fluid taken out of heart at *B*, *C* and *D*, replaced in heart. *F* = 1 c.c. of 2 p.c. frog's serum.

toxic effects, but higher concentrations of serum produced no harmful effects, and stimulated the heart for a long period. These results suggested that the active substance was used up in some way by the heart, but this important question requires further investigation.

The nature of the hypodynamic condition.

Our experiments prove that the hypodynamic heart is characterised by a diminished oxygen consumption as well as by a diminished mechanical activity, and that the substances (serum, soap, etc.) which restore the one also restore the other. The variations in mechanical activity are similar in extent to the variations in oxygen consumption.

Several workers have studied the anaerobic activity of the frog's heart and have shown that it can continue to function in an atmosphere of nitrogen for many hours. Weizsäcker(4) showed that cyanide caused a much greater reduction of oxygen consumption than of mechanical activity in the frog's heart and that this was associated with the passage of non-volatile acids into the perfusion fluid. The hypodynamic condition

might therefore be associated with incomplete oxidation and excretion of non-volatile acids (*e.g.* lactic acid) by the heart.

We tested this point by measuring the *p*H of the perfusion fluid. In one case 4 c.c. of Ringer were introduced into a heart that had been perfused for eight hours, and this fluid was left in for 15 hours. The original *p*H was 7·6 (phosphate buffer), and after perfusion the *p*H was about 7·2, but this change was due to the presence of carbon dioxide, for after passing air through the fluid for an hour the *p*H changed to 7·8. The *p*H was measured both by indicators and by the potentiometer, and in both cases there was no evidence that any fixed acid was excreted by the hypodynamic heart, but the amount of non-volatile anions in the fluid was always reduced by prolonged perfusion. This effect presumably was due to the passage of phosphate or chloride ions into the heart.

The reduction in oxygen consumption in the hypodynamic state cannot therefore be accounted for by supposing that the heart is functioning anaerobically and excreting unoxidised acid products of metabolism. The evidence available suggests that the chemical processes associated

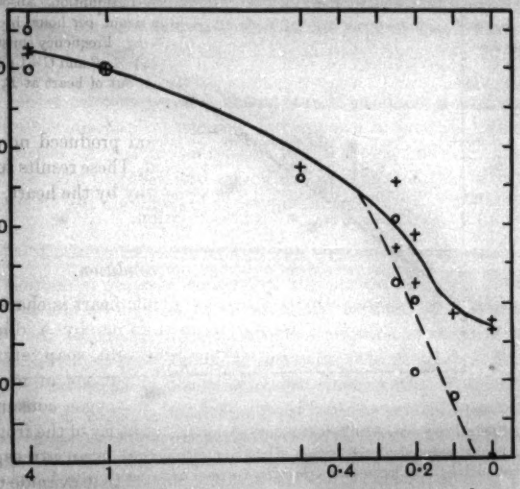

Fig. 5. Effect of variations in calcium content on mechanical response and oxygen consumption. Figures reduced to percentage of normal activity. Abscissa—millimolar calcium content. Crosses = oxygen consumption; circles = mechanical response. (Method 1, exp. 62: heart weight, 39 mg.; and exp. 65: heart weight, 64 mg.)

with contraction are similar in cardiac and in skeletal muscle; if this be assumed, then the oxygen consumption may be reduced in two ways; either by a diminution in the extent of the anaerobic break-down process or by a diminution in the extent of the aerobic recovery process. Since there is no evidence for the formation of fixed acids, we must assume that the hypodynamic condition is associated with a diminution in the extent of the anaerobic katabolic process.

C. The effect of varying calcium concentrations.

The normal Ringer's fluid had a calcium content of one millimolar, and reduction to 0·1 millimolar paralysed the heart. Fig. 5 shows the effects produced on the mechanical response and oxygen consumption by varying the calcium content of the Ringer.

The relation between the variations in mechanical response and in oxygen consumption is shown in Fig. 6. This figure records results

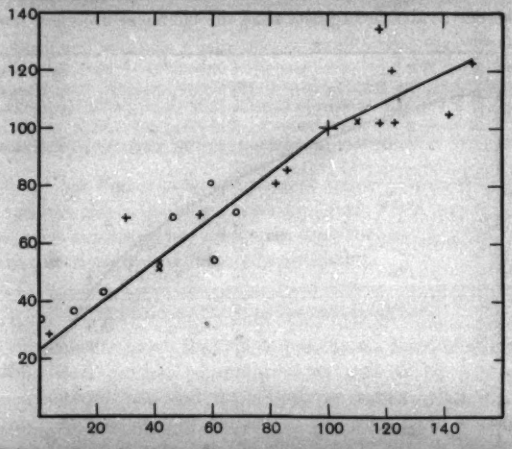

Fig. 6. Relative effect of variations in calcium content on oxygen consumption and on mechanical response. Abscissa—mechanical response; ordinate—oxygen consumption. All figures reduced to percentage of activity with normal Ringer's fluid (calcium = 2 millimolar.) The figures are taken from several experiments with different methods (o = method 1; + = method 2; × = method 3).

obtained by all three methods, and the results obtained by the different methods show a satisfactory agreement. When the heart is arrested by

lack of calcium, a resting metabolism persists which is about 30 p.c. of the normal metabolism. If this basal metabolism be subtracted, then the reduction in the oxygen consumption associated with contraction is similar in extent to the reduction in the mechanical response.

Locke and Rosenheim(5) found that there was a considerable output of CO_2 from an isolated rabbit's heart arrested by perfusion with calcium-free Locke's solution. Rohde(6) also found that the oxygen consumption of an isolated cat's heart was only reduced to a half when the heart was arrested by perfusion with Locke's solution free of calcium and potassium. These observers suggested that lack of calcium acted more powerfully on the mechanical response than on the metabolism.

Gray(7) found in the ciliated epithelium of mytilus that lack of calcium produced some reduction in the mechanical activity but did not decrease the oxygen consumption.

Our results suggest that the difference between the extent of the reduction produced by lack of calcium in the mechanical response and the reduction in the oxygen consumption in the frog's heart is due to the existence of a considerable resting metabolism, which is not affected by ionic changes. We have shown(1) that in the normal heart when contracting empty or when at rest there is a basal metabolism from 20–30 p.c. of the normal.

Increase of calcium content above normal produces a well-marked increase in the mechanical response but appears to produce rather less increase in the oxygen consumption. Fig. 6 shows, however, that the results were very variable, and we are not certain that this divergence may not be within the limits of experimental error.

D. Effects of variation in the potassium content.

The potassium chloride content of the normal Ringer's fluid was 2 millimolar and an increase to 12 millimolar arrested all movement of the heart, but only reduced the oxygen consumption to about 25 p.c. of the normal. An increase of the potassium content to as much as 30 millimolar failed to abolish this resting oxygen consumption. Similarly, the perfusion with potassium-free Ringer paralysed the heart, but an oxygen consumption about 25 p.c. of normal persisted. This effect is shown in Fig. 7. These results were obtained with all three methods. Meyerhof(8) found that potassium excess reduced the lactic acid production in the frog's sartorius, contracting anaerobically, nearly to the same extent as it reduced the isometric tension.

E. THE ACTION OF NARCOTICS ON OXYGEN CONSUMPTION.

A number of observers have shown that narcotics depress the activity of cells more than they depress the oxygen consumption. Rohde and

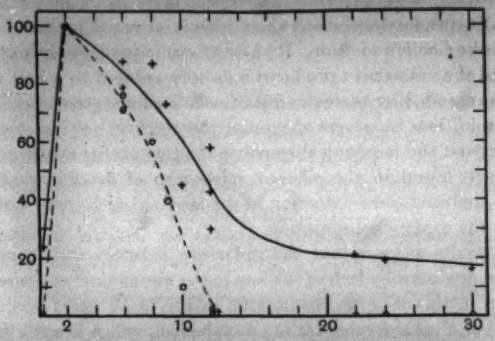

Fig. 7. Effect of alterations in potassium content on oxygen consumption and mechanical response. Figures reduced to percentage of activity with normal Ringer's fluid. Abscissæ—millimolar potassium content. + = oxygen consumption and o = mechanical response. The figure is composed of results obtained from six experiments with Method 1, and three experiments with Method 2.

Ogawa(9) found that light narcosis with chloral hydrate depressed the mechanical activity of the isolated cat's heart but produced little effect on the oxygen consumption.

Similar results were obtained by the following observers: Loeb and Wastenys(10) (fundulus embryos narcotised with chloroform); Winterstein(11) (ethyl alcohol on frog's spinal cord); Fischer(12) (ethyl alcohol on isolated cat's heart). Gray(7) found that chloral hydrate 0·5 p.c. arrested all movement in the cilia of mytilus, but that 1·1 p.c. chloral hydrate only reduced the oxygen consumption to 20 p.c. of normal. Weizsäcker(13) found in the case of the frog's heart that the concentrations of various narcotics (ethyl and amyl alcohol, ethyl and propyl urethane) required to abolish the oxygen consumption were twice as great as those sufficient to arrest the heart. Bohnenkamp(14) reported that alcohol and chloral hydrate acted chiefly in reducing the mechanical activity of the frog's heart and had little effect on the oxygen consumption. He found, however, that ethyl urethane reduced the two factors equally. Fukuda and Naito(15) found that chloral hydrate

diminished the oxygen consumption of the frog's heart more than the mechanical activity, a result that differs from those of all other workers.

In the case of skeletal muscle the results are less unanimous. Meyerhof(8) found that narcotics reduced the contractile tension in the frog's sartorius far more than they reduced the oxygen consumption. Weizsäcker(16) found that ethyl alcohol reduced the tension more than it reduced the initial heat production, but the more recent investigations of Gasser and Hartree(17) showed that alcohol produced an equal reduction in these two factors.

We measured the action of narcotics on the mechanical response and the oxygen consumption of the frog's heart by Methods 2 and 3. Fig. 8

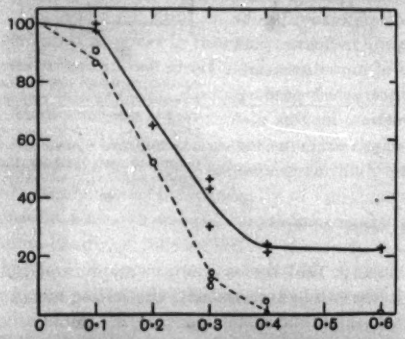

Fig. 8. Effect of ethyl urethane on oxygen consumption and on mechanical response. Abscissæ—molar concentration of ethyl urethane. The figures, which have been reduced to percentages of the normal activity, were taken from two experiments with Method 2, and two experiments with Method 3. + = oxygen consumption and o = mechanical response.

shows that increasing concentrations of ethyl urethane diminish the mechanical response of the frog's heart in a nearly linear manner, but that the reduction in oxygen consumption is not reduced below about 30 p.c. of normal by concentrations of drug three times as great as that sufficient to arrest the heart. This curve closely resembles the curve found by Gray for the action of chloral hydrate on mytilus cilia.

Other experiments with ethyl alcohol and butyl chloral hydrate gave similar results. In all cases concentrations of narcotics sufficient to arrest mechanical activity only reduced the oxygen consumption to about

30 p.c. of normal, and a considerable further increase in the concentration of the narcotic produced little further reduction in the oxygen consumption.

The relation between the reduction in the mechanical response and the reduction in the oxygen consumption produced by narcotics is shown in Fig. 9.

Our methods were not suitable for an accurate comparison between the changes produced in the mechanical response and in the oxygen use. The results obtained, however, indicate that concentrations of narcotics insufficient to produce arrest produce an equal reduction in the mechanical response, and in the oxygen consumption associated with contraction.

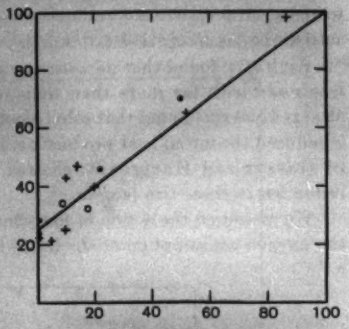

Fig. 9. Relative effect of narcotics on oxygen consumption and mechanical response. Abscissæ — mechanical response; ordinate — oxygen consumption. All figures are expressed as percentages of the normal activity. The figures are taken from experiments with Methods 2 and 3. Crosses = ethyl urethane; dots = ethyl alcohol; circles = butyl chloral hydrate.

Discussion.

We have shown (1) that the oxygen consumption of the frog's heart can be divided into two factors, namely, the resting metabolism and the extra oxygen consumption associated with contraction, and that the resting metabolism amounts to at least 20 p.c. of the oxygen consumption during moderate activity. We have also shown (1) that when the heart is paralysed by acid Ringer a metabolism persists similar in extent to the resting metabolism.

The results in our present paper show that this resting metabolism is not reduced by the hypodynamic condition, nor is it increased by the addition of serum. The resting metabolism also persists when the heart is paralysed by ionic changes (lack of calcium, lack or excess of potassium) or by narcotics. Moreover, this resting metabolism is not materially reduced by concentrations of potassium or of narcotics, which are considerably in excess of those sufficient to paralyse the heart. The resting metabolism is, however, abolished by cyanides.

Ionic changes and narcotics therefore depress the oxygen consumption due to contraction, but have little effect on the resting metabolism.

A consideration of Figs. 7 and 9 shows that variations in ionic content and in narcotics reduce the oxygen consumption associated with contraction to an extent proportional to the reduction they produce in the mechanical activity of the heart.

We were, however, only able to use small quantities of fluid when measuring both the oxygen consumption and the isometric response, and usually there was a considerable recovery of the heart during the period of an hour, which was the shortest time in which the consumption could be measured accurately. This recovery was probably due to drugs diffusing out through the walls of the heart. Consequently we were only able to obtain average values for the mechanical response and oxygen consumption. This error was greatest when weak concentrations of drugs were employed. For this reason we can only state the approximate relationship between the reduction in oxygen consumption and the reduction in mechanical response in those cases where the ionic change or narcotic concentration did not arrest the heart.

The fact that the heart can function anaerobically proves that the initial process that produces contraction is anaerobic. Our results indicate therefore that ionic changes and narcotics inhibit the initial anaerobic break-down process. We have not obtained any certain evidence that the mechanical response can vary independently of variations in this initial break-down process, although some of our results suggest that a heart in the hypodynamic condition functions more efficiently than the normal heart. This, however, is only true if comparison is made between the mechanical response and the metabolism due to contraction.

A similar increased efficiency was demonstrated by Sereni(18), who studied the H/T ratio of the skeletal muscle of the frog, when subjected to various ionic changes, and found that this ratio was decreased by lack of calcium.

The fact that the oxygen consumption of a striped muscle varies with its initial length has already led to a fairly general acceptance of the view that the initial anaerobic process in muscular contraction is located at the surface of the muscle cell. Our results indicate that ions and narcotics exert their action on this initial anaerobic process, and since there is considerable evidence that ionic changes and narcotics act on cell surfaces, our results confirm the view that the anaerobic process is located at the cell surface.

Conclusions.

1. Prolonged perfusion with Ringer's fluid reduces the oxygen consumption as well as the mechanical activity of the frog's heart.

2. Addition of serum, of alcoholic extract of serum or of soaps restores the oxygen consumption of the hypodynamic heart to normal.

3. The resting metabolism of the hypodynamic heart is as great as, or possibly greater than, that of the fresh heart, and it is not increased by addition of serum, etc.

4. Lack of calcium, excess of potassium, lack of potassium and narcotics, all reduce the oxygen consumption. The resting metabolism is not reduced, and the reduction in oxygen consumption due to contraction is proportional to the reduction in the mechanical response.

5. The resting metabolism is not markedly reduced by ionic changes or narcotic concentrations considerably in excess of those sufficient to paralyse the heart.

6. The chemical changes which cause the resting oxygen consumption appear to be of a different nature from the katabolic processes associated with contraction.

We desire to thank Prof. J. C. Drummond for his kindness in supplying us with samples of non-saponifiable fractions of oils, and Dr and Mrs Stedman for carrying out certain measurements with the potentiometer.

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NERVE EXCITATION BY MULTIPOLAR ELECTRODES.

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Introduction.

In a former paper(1) a physical theory was put forward to explain the changes of the threshold for nervous excitation which are observed when certain alterations are made in the positions of the electrodes. The assumptions were essentially that the nerve might be considered as a cylinder with a resistant sheath and a conducting core, and that the current which excited was the portion which left the core through the sheath. This portion was calculated from the known laws of current distribution, and a formula obtained which agreed in a satisfactory manner with the observations.

In a recent publication(2) the range of observations was extended to include the case of a bent nerve placed in a bath of conducting fluid between two large electrodes, but no extension of the assumptions was required for the theory to account quantitatively for the results obtained.

In the present paper the range of observations is still further extended, and a form of apparatus is used which allows of greater accuracy of measurement than in the experiments with a bent nerve. It is found that in every case the results are in complete accord with the original assumptions, and this to a degree of accuracy rather greater than has been obtained in the former experiments.

Tripolar electrodes.

If the analysis in the former two papers is accepted we must conclude that the resistance of a nerve to the passage of a transverse current is very high. From this it follows, first, that the transverse component of any current will have a negligible effect; second, that the potentials in the immediate neighbourhood of a fibre may be considered as being radially symmetrical.

These considerations in the experiments with a bent nerve justified

the calculation being made merely on the basis of the actual potential applied to the outside of the nerve fibre, and without reference to the direction of the current. The satisfactory agreement which was found between the experimental results and this calculation is a further justification of the latter.

It is clear, however, that there are many ways in which the potentials of the various points along the nerve may be made to assume a definite set of values.

For instance, the method of the bent nerve gave rise to the following potential distribution. The two extremities of the nerve which were shielded in glass tubes were equipotential regions, and the two limbs were regions of constant potential gradient. But this potential distribution may equally well be obtained by applying three electrodes to a single straight stretch of nerve. In this case the regions external to the three electrodes will be equipotential, and between the middle electrode and each of the other two will be regions of constant potential gradient.

Thus though physically the arrangement for the bent nerve is very different from that for tripolar electrodes, yet both apply to the surface of the nerve the same potential distribution, and hence both should give rise to the same experimental results.

In confirmation of this it will be seen that the formula obtained for the bent nerve may be applied exactly to the case of tripolar electrodes, and that the correspondence between theory and observation is even better here than in the former experiments.

The arrangement of tripolar electrodes with a single straight stretch of nerve has the advantage that it avoids all the difficulties which arose with the bent nerve owing to the limbs being either too slack, or else pulling on the bend too strongly and constricting it. The errors in reading the angle of the bend are also eliminated.

But there arises a new difficulty which did not occur in the former arrangement. If the electrodes are of bright metal such as silver, they will be polarisable, hence the potential of the metal will not be the same as that of the solution in contact with it; moreover the potential of the solution will in general suffer a discontinuity across each electrode. If on the other hand the electrodes are of the Ag-AgCl type and are non-polarisable, then they will have a considerable resistance due to the chloride layer on the surface, and here again the potential of the metal will be different from that of the solution in contact with it.

This discontinuity of potential complicates the mathematics somewhat, and gives rise to results which are not strictly comparable with

those of the bent nerve; but provided that the chlorination of the electrodes is very light, the discontinuity is negligible, and in this case the results are strictly comparable.

We shall consider first the experiments made with the electrodes thus lightly chlorinated.

Apparatus. The preparation (frog's sciatic-gastrocnemius) was set up in the trough used in the experiments on the determination of the relation between threshold and interpolar length (l) (Fig. 1). This apparatus was

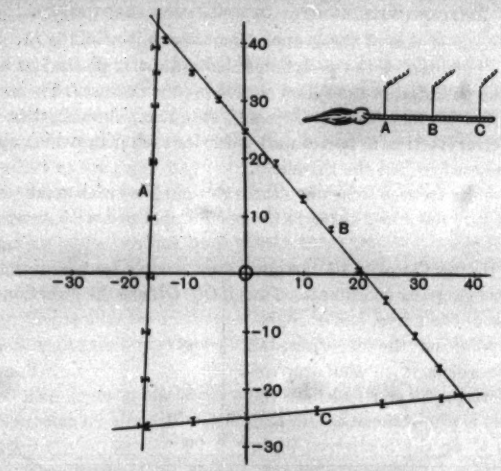

Fig. 1. Tripolar electrodes, lightly chlorinated.

modified slightly as follows. A third electrode was added, of the same form as the other two, being made of a thin sheet of silver of such a size that it exactly filled the cross-section of the trough. The other two electrodes were placed at about 30 mm. apart, and the third electrode was placed exactly half way between them. There were usually 5–10 mm. of nerve in the equipotential solution beyond the proximal electrode.

For convenience of manipulation two potentiometers were set up in parallel so that the potential of any point on the one was the same as that of the corresponding point on the other. The mid-point of one of the potentiometer wires was connected to the middle electrode of the trough, and the potential of this point is defined as zero throughout. The other

two electrodes were connected each to one of the potentiometer wires so that each could be given independently any required potential positive or negative (within limits).

The procedure was as follows. When the nerve had been set up and left to attain a steady state, determinations were made by giving to one of the electrodes a series of arbitrary potentials, and by finding for each of these what potential had to be given to the other electrode to obtain a threshold contraction.

Results with lightly chlorinated electrodes.

The result of one of these experiments is shown in Fig. 1. In this figure the potential of the peripheral electrode *A* is plotted as abscissa against the potential of the spinal electrode *C* as ordinate. The strengths are adjusted to constitute a threshold stimulus. The direction of the limits of error (vertical or horizontal) shows for each point which electrode is being altered to find the threshold.

The results are seen to lie upon three straight lines with great accuracy, and the figure resembles strongly the results obtained with a nerve bent at right angles.

Now the results of the experiments on nerves bent at right angles were plotted in polar coordinates. Thus if *OQ*, *OR* are the directions of the two limbs of the nerve, and *P* represents a point on the experimental triangular curve, then the drop of potential along the vertical limb (in the figure) is proportional to *OR*, and the drop along the horizontal limb, to *OQ*, where *ORPQ* is a rectangle. It thus appears that the threshold value represented by *P* is due to the two values *OQ* and *OR* applied to the two limbs.

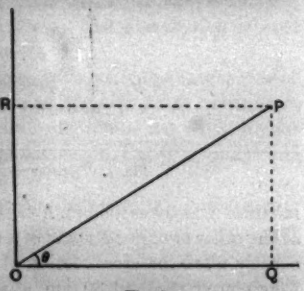

Fig. 2.

But in the experiments with tripolar electrodes, these two values *OQ* and *OR* are precisely the potentials which are applied to the extremities of the two limbs, and plotted in cartesian coordinates, whence it is seen that the threshold is again represented by the point *P*.

Therefore, as a direct result of the inefficacy of the transverse component of the current, we deduce that the results with tripolar electrodes should be identical with those where the nerve was bent at right angles.

(The reason that the tripolar arrangement is identical with the nerve bent at an angle of 90° rather than at any other angle is merely because we plot in rectangular cartesian coordinates.)

Since it has just been shown that the results are identical with the case of the bent nerve, we may adopt straightway certain conclusions obtained from those experiments, namely, that the horizontal line represents stimulation at the proximal electrode, that the vertical line represents stimulation at the distal electrode, and that the hypotenuse represents stimulation at the middle electrode.

These conclusions may all be confirmed at once by an intuitive analysis analogous to that employed in the case of the bent nerve. For instance, the hypotenuse is the only line which passes through the first quadrant, *i.e.* where both outside electrodes are positive. This means that the middle electrode is here cathodic to both the others, so that evidently the line corresponds to stimulation at the middle electrode. The other lines may be verified in the same way.

Mathematical analysis. In the Appendix of the former paper (2) a formula was obtained giving the theoretical results when the nerve was bent at an angle. As has been shown these results are immediately applicable to the case of tripolar stimulation, and they may be stated as follows:

(*a*) The results lie on three straight lines which constitute a triangle which is approximately right-angled. This is seen to be the case with great accuracy.

(*b*) The angle in the third quadrant should be not a right angle but less than this by about 6°. In the case given the angle is less than a right angle by 7°.

(*c*) The hypotenuse should be approximately symmetrical with respect to the other two sides. In the figure this line is 3° beyond the limits of asymmetry likely if the nerve is the only asymmetrical object, but since the two lateral electrodes were rather heavily chlorinated and probably to different extents, a certain amount of asymmetry would be here introduced which would probably account for the observed results. As was mentioned earlier, this experiment lends itself to greater accuracy than those with a bent nerve, and so it is very satisfactory to find that the accord with the formula is even better than in the former case.

QUADRIPOLAR ELECTRODES.

It is somewhat a matter of chance to get the required degree of light chlorination so that on the one hand the electrodes may be completely non-polarisable, and on the other so that the chloride layer may not be appreciably resistant. It is easy, however, to set up an arrangement which amounts to four non-polarisable electrodes without any layer of resistant material.

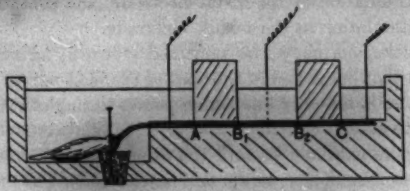

Fig. 3. Apparatus: quadripolar electrodes.

Apparatus (Fig. 3). The apparatus was almost identical with that used in the former experiments, and the only essential difference was as follows. Two blocks of ebonite, fitting exactly the cross-section of the trough, were inserted one on either side of the middle electrode. Each block was 10 mm. in length, and in section occupied the whole trough with the exception of the small groove at the bottom which contained the nerve. It will be seen that with this arrangement nearly the whole drop of potential between the middle electrode and one of the other ones will occur in the small groove under the block, and hence, to a first approximation, we may regard the parts of the nerve which are under the blocks as being in regions of constant potential gradient, whereas the other parts are in equipotential solutions.

The case is therefore that of quadripolar electrodes, where the electrodes must be considered as situated at either end of the two blocks. In this arrangement the "electrodes" have not the disadvantage of polarisation etc. previously discussed, and the metallic electrodes where polarisation is still occurring are now in the equipotential regions and at some distance from the various seats of excitation.

In order that the middle equipotential region should be at the same potential at either side of the middle electrode, this electrode was not a silver plate, as in the former experiments, but a silver grid, so that there was a free passage of the electrolite from one side of it to the other.

If we assume that essentially all the potential drop occurs in the

narrow regions under the blocks, the potential applied to the nerve will be as represented in Fig. 4.

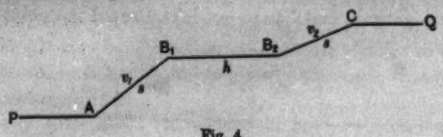

Fig. 4.

Applying the general formula from the former paper, and approximating as before, we obtain

$$\text{at } A \qquad 2\frac{\partial^2 V}{\partial x^2} \propto v_1 + v_2 e^{-s-h},$$

$$\text{at } B \qquad 2\frac{\partial^2 V}{\partial x^2} \propto v_2 e^{-h} - v_1;$$

where the constants of proportionality do not involve v_1, v_2, or h.

The values of $\frac{\partial^2 V}{\partial x^2}$ at B_2 and C are analogous to B_1 and A.

It thus appears that the results should lie upon a quadrilateral, one line corresponding to excitation at each of the four "electrodes."

With regard to the directions of these lines, it is easy to see from the formula that the lines corresponding to excitation at A and C lie in the same directions as in the former experiments. The other two lines diverge at an angle dependent upon h. When h is very small the two lines coincide, when h is very large they diverge at a right angle.

These relations are unaltered by the fact that the quantities actually plotted are not v_1 and v_2, but values which are proportional to these.

Experimental results.

Fig. 5 shows two sets of results taken from the same preparation, but with different lengths of the middle equipotential region. The analysis is the same as in the former cases. The two lines corresponding to excitation at the lateral electrodes are but slightly affected by the alteration of h, but the deviation of the other two lines is considerably changed.

In Fig. 6, where h is still further increased, the deviation approaches the theoretical maximum of a right angle. In this figure it is seen that these two lines do not continue strictly straight until they meet, and in this respect they may be contrasted with the opposite angle of the quadrilateral. The effect, which naturally comes into prominence only when the deviation is great, is certainly an experimental fact, since both in this and in other similar experiments I have carefully redetermined all the

adjacent points on the curve. Of the many possible explanations which present themselves, nothing seems sufficiently obvious and simple to be accepted straightway, and I have not investigated the point further.

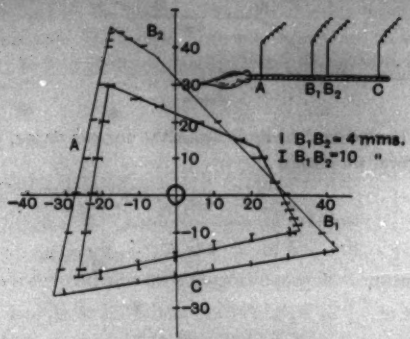

Fig. 5. Quadripolar electrodes.

On the whole, the experiments with quadripolar electrodes correspond in a satisfactory manner with the results of the theory, and it is seen that,

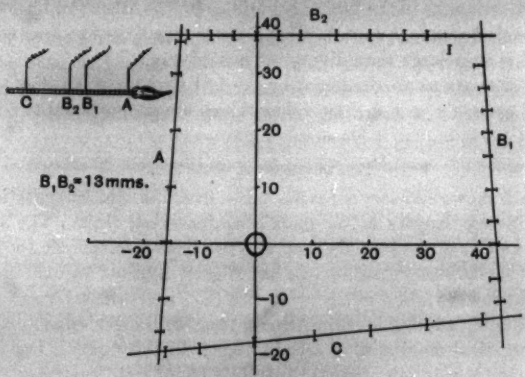

Fig. 6. Quadripolar electrodes.

with the exception of the point just mentioned, there is a single straight line corresponding to stimulation at each electrode, so long as there is no discontinuity of potential at the electrodes.

Potential discontinuous at the electrodes.

It is hardly worth while analysing in detail the cases where the potential suffers a discontinuity at the electrodes either due to polarisation or resistance. But it may be of interest to note what changes in the curves are to be expected in this case, and to observe that these changes are just those which occur.

The heavily chlorinated electrodes will have deposited upon their surfaces a layer of very high specific resistance, and so, although it is thin, it will have an appreciable resistance. As a result, the potential applied to the nerve from tripolar electrodes will assume the form shown in Fig. 7.

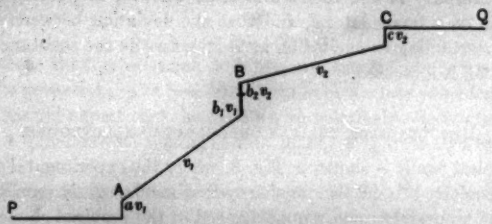

Fig. 7. Applied potential with tripolar electrodes.

The results may easily be worked out in detail from the general formula derived in a former paper (2), but it will be sufficient in the present instance to consider the matter intuitively.

It is seen that at each electrode the potential on the outside of the resistant sheath of the nerve suffers a discontinuity, but the potential on the inside of the sheath is continuous. It follows that the current leaving the nerve through the sheath, *i.e.* the current which stimulates, is greater on one side of the electrode than the other. But since we observe only the threshold, we deal only with that point on the nerve where the stimulating current is greatest in each case. Thus, if we excite at *A* as in Fig. 7, since the potential towards *P* is more negative than the potential towards *B*, the current leaving through the sheath will be greater on the side towards *P* and we need not consider the value on the other side. In the same way *C* will excite on the side nearer *Q*. In the case of *B* we should expect from symmetry that excitation would not take place always on one side or always on the other, and hence in all there would be four places whence excitation could be elicited,

A and *C* on the sides remote from *B*, and *B* on either side.

To these intuitive conclusions may be added some results from a more detailed study of the mathematics.

If the experimental results are plotted as before, they should lie upon a quadrilateral, one side of which corresponds to each of the four places whence excitation may be elicited.

The lines corresponding to *A* and *C* are in much the same direction as in the former cases. The deviation between the other two lines will be greater the greater the discontinuity at the electrodes, and will be very small when the discontinuity is small, as has been shown at the beginning of this paper.

We may now compare the experimental results and note that whether the discontinuity is due to resistance, or whether to polarisation, the results lie on a quadrilateral, and that the deviation between the two lines corresponding to excitation at *B* increases as the resistance or the polarisation is increased.

Results with heavily chlorinated electrodes.

A typical result is shown in Fig. 8, where the experimental observations are plotted as in the previous experiments. It is seen that the results lie accurately upon a quadrilateral of the required shape.

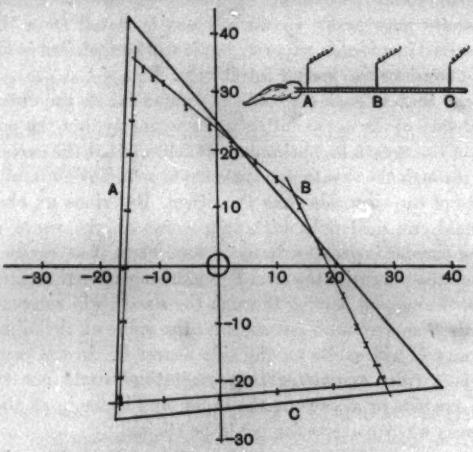

Fig. 8. Tripolar electrodes, heavily chlorinated.

In this actual experiment the nerve is the same as that used in Fig. 1, and the electrodes *A* and *C* are identical both in position and with respect to their chloride coats. The only difference is that between the two sets of observations the middle electrode was taken out, and a heavy layer of chloride deposited upon both sides of it. The electrode was then replaced as before.

In order to facilitate the comparison of the two figures, the three lines of Fig. 1 are also included in Fig. 8.

It is seen that alteration of the resistance of the middle electrode has but little effect upon the directions of the lines corresponding to excitation at the other two electrodes, but that it causes the hypotenuse of the original triangle to break up into two lines which exhibit a marked deviation.

Effect of polarisation.

It is clear that polarisation will produce exactly the same discontinuities in potential as the resistant chloride coats just considered, and hence we should expect that the results with polarisable electrodes would fall upon a quadrilateral similar to that in Fig. 8. This is seen to be the case.

Typical results are given in Figs. 9 and 10. Fig. 9 represents three

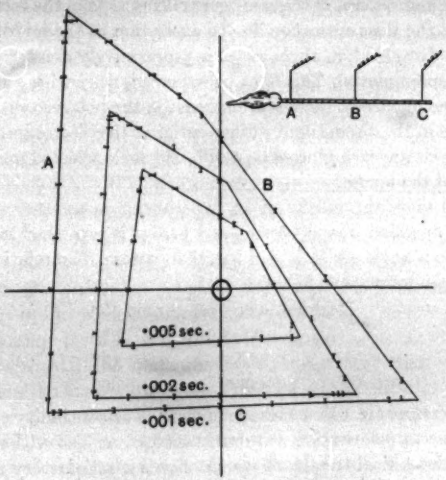

Fig. 9. Polarisable tripolar electrodes.

determinations made upon the same preparation in which the duration of current was approximately ·001, ·002, and ·005 respectively. It is seen

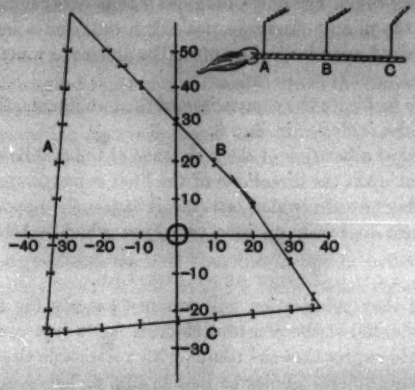

Fig. 10. Polarisable tripolar electrodes. Duration ·0005 sec.

that the results are qualitatively exactly as predicted. In each case they lie upon a quadrilateral of the same general shape as in the former experiments, and the sides corresponding to excitation at the middle electrode are seen to diverge at an angle which is progressively greater, the greater the duration employed. This is to be expected, since with an increase in the duration of current there is an increase in the polarisation, and hence an increase in the discontinuity of potential at the electrodes. (The three quadrilaterals are each plotted on a different scale selected merely for the clearness of the figure.)

Fig. 10 gives the results of an experiment on another preparation where the duration was ·0005 sec., and here it is seen that the two lines diverge at an angle which is still less than before. The relation between angle and deviation is as follows:

Duration	·005	sec., angle	33°
,,	·002	,, ,,	25°
,,	·001	,, ,,	15°
,,	·0005	,, ,,	9°

It is therefore clear that these conditions of discontinuity of potential, whether due to polarisation or to resistance, come also within the scope of the mathematical treatment which affords a satisfactory account for the observations obtained.

Conclusions.

In the many theories of nervous excitation which have been put forward, the stress has sometimes been laid upon the structure of the tissue, as in the "Kernleiter model," and sometimes upon the generalised physical principles which are supposed to underlie the excitatory process, as in Nernst's theory. A complete theory must, clearly, combine these two aspects, and the present work was undertaken in the hope that some information might be gained as to the place in the nerve fibre where Nernst's polarisation occurred.

Unfortunately this question has not been much advanced. There seems good ground for supposing that the membranes of Nernst are cylinders, coaxial with the nerve, and that the current stimulates as it leaves the nerve, but these experiments give no information as to whether the membranes are within the medullary sheath or outside it, whether they are single as Nernst supposed, or double as Hill suggested, and far less can we identify them with any membrane visible through the microscope.

In one respect, however, the experiments give a very definite result. If these cylindrical membranes are to have any place in the visible histological structure of the nerve it seems likely that they must be located as bounding the medullary sheath either inside or outside, and hence the stimulating current will have to pass through this very resistant sheath to excite. Now the amount of current which will pass through a resistant sheath of this kind will depend upon the applied potential all along the nerve and may be worked out from the physical principles underlying current distribution. This has been done, and the results applied to several different kinds of potential distribution. In every case it has been found that, in order to obtain a threshold excitation, the values of the potential at various points along the nerve have to be so adjusted experimentally that the current leaving through the sheath at the point where excitation is occurring remains always at the same value.

This on the one hand supports the suggestion that the excitable membranes are in close proximity to the medullary sheath so that the current passing through the membranes is the same as that passing through the sheath, and it affords very strong evidence that we are right in supposing the membranes to be cylinders coaxial with the nerve.

On the other hand it supplies a simple and comprehensive explanation of the variation of threshold with the position of the electrodes. A formula is derived which is easily applicable to all cases provided that the electrodes are large compared with the diameter of a nerve fibre, and

that the potential applied to each point of the nerve is known, and this formula fits the observations with an accuracy as great as the accuracy of the experiment.

The following is a list of the ways in which the applied potential has been altered; in every case the resulting change in threshold is quantitatively as was predicted by the formula.

1. Variation of interpolar length between bipolar electrodes.
2. Variation of angle between current and nerve (single stretch).
3. Variation of angle between current and nerve (bent nerve).
4. Nerve as in 3 but constricted at bend.
5. Tripolar electrodes, lightly chlorinated.
6. Tripolar electrodes, heavily chlorinated.
7. Polarisable tripolar electrodes.
8. Non-polarisable quadripolar electrodes.

As a result of these experiments it seems justifiable to conclude that when the threshold alters as the result of a change in the space arrangement of the applied potential, this alteration may be interpreted quantitatively on the basis of the foregoing theoretical considerations.

Summary.

In a recent paper (2) a general mathematical formula was obtained to explain on some very simple assumptions the effect of the position of the electrodes upon the threshold for nervous excitation.

The present paper shows that the predictions of this formula are fulfilled in several different cases, namely:

Tripolar electrodes which are polarisable, lightly chlorinated, heavily chlorinated;

Quadripolar electrodes which are non-polarisable.

In conclusion I wish to express my thanks to Dr Adrian for his valuable criticism.

I am also indebted to the donor of the Stokes Studentship.

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SOME FACTORS INVOLVED IN THE CONCENTRATION OF BLOOD BY THE SPLEEN.

By J. BARCROFT and H. W. FLOREY.

(*From the Physiological and Pathological Laboratories, Cambridge.*)

It has been shown by Barcroft and Poole(1) that it is possible to abstract from the spleen pulp blood which is more concentrated in hæmoglobin and corpuscles than that taken at the same time from the peripheral circulation; that is to say the spleen is capable of concentrating blood contained within it.

There are three possible explanations for this: (1) that corpuscles are manufactured in the spleen and added to the blood circulating through it, but all evidence is against the idea that the spleen in adult life manufactures corpuscles at all; (2) that the blood for some reason is held up in the spleen and during its sojourn the fluid is filtered off from it into spleen lymphatics, the corpuscles remaining in the pulp spaces. The samples then obtained by puncture of the pulp spaces will obviously be more concentrated than peripheral blood; (3) that the peculiar anatomical arrangement of the vascular bed in the spleen may favour a form of plasma skimming whereby corpuscles are concentrated through removal of plasma by the blood stream.

If the last explanation be correct, then shutting off the main venous return should bring any process of concentration to a standstill. If however the spleen be furnished with an apparatus for removing fluid, *i.e.* lymphatics, then shutting off the main veins, owing to increased intravascular pressure, should lead to a greater removal of fluid and concentration of blood.

These ideas have been put to the test in the following way. Cats anæsthetised with chloralose were used throughout. The spleen was exposed through an abdominal incision and blood samples taken in the way previously described. Peripheral samples were drawn from the saphenous vein into a dry syringe, hæmoglobin estimations being made on the specimens thus obtained.

After comparable specimens of spleen and peripheral blood were taken the spleen was made to contract by direct faradic stimulation in order to expel its blood content. The organ was then divided into two

portions by means of a thick ligature and the main vein from one portion tied. After a lapse of time further pulp specimens were abstracted from congested and normal portions respectively.

In five such experiments a considerable concentration invariably appeared in the blood of the congested half.

Protocols.

Cat. Chloralose. November 5, 1927.

a.m.		Hb. p.c.
10.40	Saphenous vein sample	66
10.50	Spleen sample before vessels tied. Spleen appeared purplish blue colour ...	83
10.59	Spleen sample after spleen tied into two portions but before main vein tied. Spleen changed from purple to red and became smaller	69
11.4	Vein tied off. Spleen immediately swelled	
11.19	From tied off portion of spleen	58
11.23	From tied off portion of spleen	73
11.27	Saphenous vein sample	61
11.34	From tied off portion of spleen. Artery held during taking of sample ...	90
11.47	Saphenous vein	64
11.52	From tied off portion of spleen. Artery held	85
11.57	From tied off portion of spleen. Artery held	98
p.m.		
12.5	Blood from portion of spleen with open vein. Spleen was rather contracted and blood difficult to obtain	67

Cat. Chloralose. October 21.

a.m.		
10.47	Spleen specimen	69
10.53	Saphenous vein sample	67
11.3	Saphenous vein sample	66
11.10	Spleen sample	83
11.38	Spleen tied into two portions with thick ligatures. Ligature placed round main vein but not tied. Spleen made to contract by direct stimulation with electrode. Puncture immediately after but no blood obtained	
p.m.		
12.0	Spleen sample obtained. Vein then tied	72
12.14	Spleen sample from tied off portion	86
12.17	Spleen sample from portion with vein not tied off	67
12.46	Spleen sample from tied off portion	94
12.48	Spleen sample from untied off portion	74
1.0	Saphenous vein sample	67 65

These experiments show that the spleen in a state of venous congestion can concentrate blood. The obvious channels therefore for the removal of fluid are the lymphatics.

Lymphatics from the spleen have been described (3, 4). There are superficial collecting trunks which are well developed in the ox and horse. In these animals they form a rich network which is situated between the

peritoneum and the fibrous capsule of the spleen. They then run towards the hilum of the spleen and terminate in the same way as the deep collecting trunks. The deep collecting trunks are connected to the preceding by numerous anastomoses. They are satellites of the blood vessels. In the hilum they are reduced to from 6–10 trunks which end in the external glands of the spleen chain.

A perfectly well-defined lymphatic system draining the spleen is therefore accepted by anatomists. It appeared to us desirable nevertheless to demonstrate, if possible, the elimination of fluid from the spleen by its lymphatics.

In line with the conception of this method of drainage the following observation was made in some of the experiments on venous ligature. After the spleen had been congested for some time it was possible to see, when pressure was exerted on the base of the splenic mesentery, blood-stained lymph eddying into the clearer fluid of the cisterna chyli. This observation has also been made by Kölliker. In this connection it may be mentioned that in some cats one meets with a small lymphatic gland in apposition to the splenic vessels near the hilum.

The following method also has been resorted to. With the object in view of perfusing the organ with all venous return blocked, the arterial and venous anastamoses with vessels of neighbouring structures were carefully ligated and cut, leaving the main artery and vein alone patent. A canula was inserted into this artery as close to the spleen as practicable, and the vessel was ligated near its exit from the aorta. The vein was tied. An artery and vein running in the splenic mesentery and supplying the upper pole of the spleen were ligated. One p.c. trypan blue solution at 130 cm. H_2O pressure was then run through the arterial canula. The spleen swelled up with this solution since the main vein was tied. In some experiments, after a short interval of time it was possible to see a blue coloured lymph entering into the cisterna chyli and mixing with the clear or white fluid there. This blue fluid emerged from the splenic mesentery. It had passed into the lymphatic system from the spleen, fatty tissue near it or from both. In some experiments however this phenomenon was not observed, a possible explanation being the ligation or rupture of lymphatics during the manipulations necessary for the insertion of the arterial canula and tying of veins. In one experiment of this type a perfectly conclusive result was obtained, for it was possible to trace a vessel filled with blue solution from the splenic hilum to the gland at the base of the mesentery. The general direction of the vessel was that of the main artery and vein, though it was not in close apposition to them.

From this gland several more blue filled trunks were followed to a second and then to the cisterna chyli.

It appears therefore that fluid can be eliminated from the spleen by lymphatics under the conditions of our experiments.

It may be objected that during life the splenic vein is not occluded and in consequence this drainage may not be of much significance. Certain observations nevertheless are in favour of the conception that the splenic veins are partially shut down under normal conditions. Tait and Cashin(2) have called attention to the very great contractility of these vessels when electrically stimulated. We have observed a considerable contraction from mechanical stimulation while clearing the vein as a preliminary to ligation.

That the blood does stagnate in the spleen is evidenced by the following observations. If the spleen of a chloralosed cat be exposed through a small incision it will usually be large and of a dark bluish-black colour, the blood in the veins being dark blue. When the spleen is made to contract by handling, electrical stimulation or sometimes merely by the insertion of a needle into its pulp, the colour immediately changes to a bright arterial red and the blood in the veins is now a bright red colour.

Whether the idea that the venous side of the splenic circulation has the importance here suggested awaits further investigation.

Summary.

(1) Possible factors enabling the spleen to concentrate its contained blood are discussed.

(2) It is shown that ligation of the venous channels causes considerable concentration of the splenic blood.

(3) Evidence is produced that fluid is drained away by lymphatics under such conditions.

(4) A suggestion is made that the very marked contractility of the splenic vein is a factor of importance in connection with the phenomena discussed.

We are indebted to the Government Grant Committee of the Royal Society for defraying the expenses of the above research.

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THE EFFECT OF OXYGEN LACK ON SURVIVING SMOOTH MUSCLE.

By R. C. GARRY.

(*From the Department of Physiology and Biochemistry, University College, London.*)

It is often tacitly supposed that an inadequate supply of oxygen causes a fall of tone and a cessation of movement in surviving smooth muscle. Yet, in physiological literature, there is no unequivocal evidence in support, indeed there is even direct contradiction in the findings of separate workers.

Mikulicz-Radecki and Lueg(12) found that interference with the blood supply of the uterus *in situ* caused a general diminution in rhythmical movement and, in some cases, a fall in tone. By a similar procedure Schindler(16) found a relaxation of the uterine musculature, but considered this effect due either to lowering of oxygen tension or to rise of carbon dioxide content of the cyanotic blood. Hoskins and Hunter(4) found that the rat intestine showed greater tone when in the presence of oxygen, but concluded that variations in the mechanical disturbance by the bubbling gas played no small part in this result. Gross and Clark(3) found that mere stoppage of the oxygen supply to the excised surviving tissue, with or without the replacement of the mechanical factor by means of an indifferent gas, caused a rapid fall in tone in the rat and rabbit intestine, but that the pendular movements persisted much longer.

Magnus(11), however, found that the circular muscle of the cat's bowel responded to absence of oxygen by a gradual increase in tone, while the longitudinal muscle coat gradually relaxed. In this case also there was no replacement of the oxygen supply by a neutral gas, so that the development of definite oxygen lack was of slow onset and was complicated by cessation of the mechanical disturbance due to bubbling. Kehrer(6), too, found that simple cessation of oxygen supply caused relaxation of uterine muscle *in vitro*, but that, when the tissue was *in situ*, interruption of blood supply caused a transient tonic contraction.

On the other hand Johnson, McClosky and Voegtlin(5) found

that very complete replacement of oxygen by nitrogen caused a practically invariable rise in the tone of excised surviving guinea-pig uterus and rabbit intestine.

How far the above findings and others scattered through the literature are comparable is, of course, a moot point. It is obvious that any investigation of this question must take into consideration the fact that the oxygen consumption of smooth muscle is low—of the order of 0·5 c.c. per grm. per hour (8)—so that the lack of oxygen must be as abrupt and as nearly complete as possible in order to obtain reliable results. Mere cessation of oxygen supply to the tissue in a saline bath, apart from the objection that it removes a mechanical stimulus, is not sufficient to ensure a critical lack of oxygen within a reasonable period of time. There is also the possibility that there may be, as suggested by Johnson, McClosky and Voegtlin, a dehydrogenation process within the tissue which may itself be capable of masking an oxygen lack of relatively short duration.

Methods.

The usual type of bath for recording movements of excised visceral muscle was used, but in the outer water jacket there were two inner vessels, each of 100 c.c. capacity, and a saline reservoir for each vessel. Thus two similar portions of tissue, *e.g.* the two horns of the same uterus, could be studied simultaneously under exactly similar conditions. From this initial state the environment of one piece of tissue could be altered and the behaviour of the companion piece of muscle used as a very necessary control of responses from the tissue under investigation. Whenever the same treatment was meted out to both tissues at the same time the result was invariably similar.

The tissues were always attached to balanced frontal-writing levers by a human hair, and a slight known tension (about 0·5 grm.) applied to the tissue.

Saline solutions of varying composition were employed. The fluids were of the following composition:

Phosphate Ringer: NaCl, 0·85 p.c.; KCl, 0·042 p.c.; $CaCl_2$ (anhyd.), 0·024 p.c.; Na_2HPO_4 (anhyd.), 0·06 p.c.; molar H_3PO_4, 0·02 c.c. p.c. to 0·06 c.c. p.c.; glucose, 0·1 p.c.

The hydrogen ion concentration of this fluid varied with the content of H_3PO_4, the range being from *p*H 7·1 to 7·3.

Phosphate Tyrode: NaCl, 0·8 p.c.; KCl, 0·02 p.c.; $MgCl_2$ (anahyd.), 0·01 p.c.; $CaCl_2$ (anahyd.), 0·02 p.c.; Na_2HPO_4 (anahyd.), 0·06 p.c.; molar H_3PO_4, 0·06 c.c. p.c.; glucose, 0·1 p.c.

The hydrogen ion concentration of this fluid was relatively high, *p*H 7·1, but, at any lesser concentration of hydrogen ions, the calcium precipitated out with disconcerting ease.

Bicarbonate Ringer: NaCl, 0·9 p.c.; KCl, 0·042 p.c.; $CaCl_2$ (anhyd.), 0·024 p.c.; $NaHCO_3$, 0·02 p.c.

Bicarbonate Tyrode: NaCl, 0·8 p.c.; KCl, 0·02 p.c.; $MgCl_2$ (anhyd.), 0·01 p.c.; $CaCl_2$ (anhyd.), 0·02 p.c.; NaH_2PO_4 (anhyd.), 0·005 p.c.; $NaHCO_3$, 0·1 p.c.; glucose, 0·1 p.c.

As the two latter solutions altered in *p*H with the tension of CO_2 of the environment, the great majority of experiments were carried out in phosphate Ringer. In some few cases a solution of the composition recommended by Johnson, McClosky and Voegtlin was used, but without any different results.

Lack of oxygen was effected by replacing the stream of oxygen bubbles by an equal stream of nitrogen bubbles. The commercial nitrogen from the cylinder contained about 0·5 p.c. of oxygen; to remove this completely, the gas was passed through a metre-long column of an alkaline solution of sodium hydrosulphite ($Na_2S_2O_4$) as described by Kautsky and Thiele(7). Thereafter the gas was thoroughly washed and led, by means of glass tubing, to the vessel containing the preparation.

The effectiveness of the purification was repeatedly tested by analysis of the gas in a Haldane atmospheric CO_2 analysis apparatus converted, by the addition of a "pyro" burette, to analyse for small amounts of oxygen. Within the limits of the graduations (1 part in 10,000) the nitrogen as used was free from oxygen.

There was, however, the remote possibility of diffusion of oxygen from the air into the fluid. A layer of liquid paraffin over the fluid might impart a false sense of security, but in any case had obvious disadvantages, so in order to exclude air the mouth of the vessel was closed with a tight-fitting rubber stopper, and the fine hair from the preparation to the lever passed through a pin-hole aperture in a glass tube inserted through the rubber bung. The rate at which the gas entered the vessel was sufficient to ensure a stream of nitrogen at a velocity of the order of 1 m. per sec. outwards through this small hole. Analysis of the gas over the surface of the fluid showed that the measure was effective, since, shortly after admission of nitrogen, all measurable oxygen had disappeared from the vessel. In a previous, somewhat similar, series, hydrogen, instead of nitrogen, replaced the oxygen, and here analysis of the saline solution by Winkler's method showed a drop of oxygen content from 1·63 p.c. to 0·08 p.c. within twenty minutes of replacement of the oxygen by hydrogen.

A few records were obtained by using the Trendelenburg(13) technique for small bowel. In this apparatus records are taken simultaneously, one of the pendular movements, the other of the alterations in the volume of the contents of the bowel. The former trace depends mainly on the condition of the longitudinal muscle coat, the latter mainly on the state of the circular muscle coat. If the fluid inside the bowel exerts a pressure of from ten to fifteen millimetres of water, regularly recurring bursts of peristaltic waves can be elicited from a preparation of guinea-pig gut. The usual small bottle, between bowel lumen and volume recorder, was replaced by a vacuum-jacketed flask in order to eliminate the effects of alteration of environmental temperature on the contained air.

The tissues used were virgin and non-virgin guinea-pig uterus, pregnant and non-pregnant cat uterus, and also the uterus of the bitch. Rabbit, cat and canine gut were used in the usual form of apparatus, while guinea-pig and rabbit jejunum were used in Trendelenburg's apparatus. Systemic and coronary arteries were employed after the manner of Cruickshank and Subba Rau(1), and in a few cases the retractor penis of the dog was used.

Results.

Most tracings from active surviving smooth muscle can be described under the headings of general tonicity, with its variations, and of amplitude and frequency of the rhythmical movements superimposed on this basal state of tonus. This arbitrary classification has at least the merit of simplicity, and moreover it seems not improbable that contraction and tonus are the expressions of fundamentally different processes.

Tonus. In the initial experiments of this series, before all trace of oxygen had been removed from the nitrogen, there was not infrequently little or no alteration in the tone level of the preparation when the stream of oxygen was replaced by the imperfectly purified nitrogen. This was most noticeable in preparations of virgin guinea-pig uterus, where the tone normally is very low. Most bowel preparations, however, were more sensitive to oxygen lack and showed an obvious fall in tone even on exposure to commercial nitrogen.

Later, however, when the nitrogen had been more thoroughly purified, all preparations, with one or two exceptions in a series of over one hundred, showed an obvious loss of tonicity whenever the oxygen want became acute. The rapidity of this fall varied in different preparations, uterine preparations usually showing a more rapid rate of fall than bowel preparations.

In two experiments with a strip of cat's uterus preserved over night in the ice room, the absence of oxygen caused an initial fall in tone followed by a slow and sustained rise in tonicity (Fig. 2 *A*).

Fig. 1. *A*. Cat uterus, effect of oxygen lack. *B*. Cat bowel, effect of oxygen lack. Note initial rise in tone on admitting nitrogen. *C*. Rabbit bowel, effect of oxygen lack. Note stimulant action of methylene blue in oxygen, but its inability to prevent action of nitrogen. *D*. Virgin guinea-pig uterus, effect of oxygen lack. Note initial low tone, absence of rhythm in nitrogen, subsequent rise in tone, and failure of S—S glutathione to alter tone in nitrogen. Time traces in minutes.

Immediately after replacement of oxygen by nitrogen there was often a transient rise which rapidly gave place to a sustained fall in tone. This was best seen in preparations from cat bowel. Gross and Clark (2) have already noted and illustrated this point (Fig. 1 *B*). In no case, however, with the exceptions given above, was there observed a sustained rise of tone such as Johnson, McClosky and Voegtlin described.

In the Trendelenburg apparatus exclusion of oxygen caused an increase in the contents of the excised bowel segment and a lengthening of the entire preparation. Magnus (11) found that the circular and the

longitudinal coats reacted differently to lack of oxygen, but, in this series, far from the circular fibres contracting and the longitudinal fibres

Fig. 2. *A.* Cat uterus, effect of oxygen lack. Note initial fall and subsequent rise. (One of the exceptional cases.) *B.* Rabbit uterus, effect of oxygen lack. Note initial fall on admission of oxygen. *C.* Bitch uterus, effect of oxygen access followed by oxygen lack. *D.* Non-virgin guinea-pig uterus, effect of admission of oxygen after a period of anoxybiosis. *E.* For comparison with *D.* Same preparation, effect of "Perhydrol" in presence of nitrogen. *F.* Pregnant cat uterus, effect of oxygen lack. Note action of adrenaline in oxygen and absence of action of adrenaline in nitrogen. Time traces in minutes.

relaxing, all fibres relaxed, the volume change often being apparently great in comparison with the lengthening of the preparation. This relaxation of circular fibres (Fig. 3 *B*) was very obvious to the naked eye.

On re-admission of oxygen the tone ultimately invariably rose. An attempt to correlate the interval of time, between oxygen admission and the onset of rise in tone, with the duration of the antecedent exposure to nitrogen gave equivocal results. This restoration of tone could be elicited also in the presence of nitrogen by the addition of minute amounts of hydrogen peroxide ("Perhydrol") to the bath (Fig. 2 *E*). The oxygen liberated from the amounts used would be more than enough temporarily to saturate the fluid with oxygen.

In certain preparations the admission of oxygen gave an initial transient fall of tone, a fall which rapidly gave place to a sustained contraction (Fig. 2 *B*).

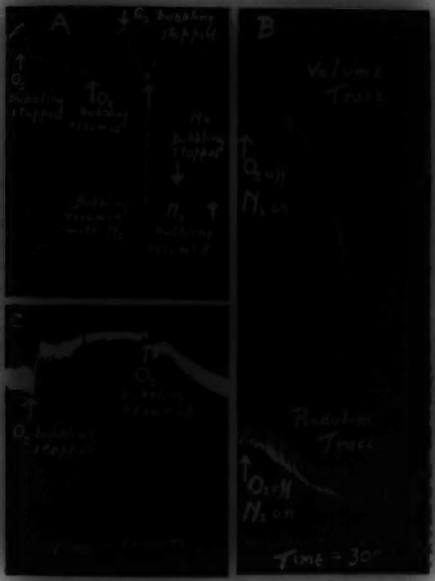

Fig. 3. *A*. Cat uterus, effect of mechanical disturbance in oxygen and in nitrogen. *B*. Rabbit bowel in Trendelenburg's apparatus, effect of oxygen lack. Note persistence of rhythm, its greater amplitude and irregularity in nitrogen. *C*. Rabbit bowel, effect of absence of mechanical disturbance (cf. *A*). Unless otherwise stated, time trace in minutes.

Rhythmical movements. In the great majority of tissues rhythmical movements disappeared in the total absence of oxygen. The uterus of the guinea-pig, however, in the presence of nitrogen with a trace of oxygen present, tended to show a large rhythmical alteration in tone at an average level of tonus somewhat below that exhibited in pure oxygen. This appearance became less frequent the more successful our attempts at purification of the nitrogen. Compare, in this connection, Fig. 4 *A*, where the nitrogen contained a trace of oxygen, with Fig. 1 *D*, where the nitrogen was pure. The tissue was virgin guinea-pig uterus in both cases.

The rabbit bowel, however, often showed a determined persistence of a fine rhythm even with nitrogen which, within the limits of our analysis,

was free from oxygen. Such fine movements eventually ceased as the exposure continued (Fig. 6, first part). In Trendelenburg's apparatus, too, peristaltic, segmental and pendular movements persisted for a long

Fig. 4. *A*. Virgin guinea-pig uterus 100 minutes in nitrogen with a trace of oxygen. *B*. Rabbit bowel in Trendelenburg's apparatus, persistence of rhythm. *C*. Non-virgin guinea-pig uterus, effect of oxygen lack and effect of NaCN. *D*. Mesenteric arteries, effect of adrenaline in nitrogen and in oxygen. Time traces in minutes.

time in the absence of oxygen (Fig. 4 *B*). Ultimately these movements also diminished in strength, the contractions becoming incoordinate and irregular. In Trendelenburg's apparatus there is always the possibility of access of oxygen from the Tyrode fluid inside the bowel lumen itself.

The substitution of oxygen for nitrogen often caused a transient quickening of the rhythmical movements.

Mechanical factors. In investigations with smooth muscle there looms largely the question of the possible importance of the effect on tone and rhythm of the mechanical disturbance caused by the bubbling gas. Direct experiment shows it to produce no effects comparable with those induced by presence or absence of oxygen. Stoppage of the stream

of oxygen bubbles, without replacement by another gas, usually caused a slight fall in tone with unaltered rhythm (Fig. 3 *A*). Sometimes, however, cessation of bubbling caused a rise in tone (Fig. 3 *C*). When in nitrogen simple interruption of the stream of nitrogen bubbles made not the slightest difference to the behaviour of the preparation (Fig. 3 *A*).

The action of drugs. Those drugs which normally lower tone in smooth muscle cannot be expected to show activity if the tissue already has a low tone in nitrogen. Those drugs which normally raise tone may be able to give some little information about the state of a tissue with low tone in nitrogen. Nevertheless the information which drugs can give us is limited, for, not only is our knowledge of the mode and site of action of drugs extremely indefinite, but also, under such abnormal conditions as lack of oxygen, we have no means of discovering whether the oxygen lack primarily affects the drug itself, a not inconceivable postulate, or some element in the nerve-muscle complex casually called smooth muscle.

Cyanide (NaCN), even in very dilute solution, gave a fall in tone and a cessation of rhythmical movement comparable with that induced by oxygen lack (Fig. 4 *C*). In oxygen the effect of NaCN gradually wore off. In nitrogen NaCN gave no result, because of the already low tone, but it prevented the restoration of tone on the re-admission of oxygen.

Pituitary extract, in complete absence of oxygen, had no effect on the guinea-pig uterus, but in the presence of a minute trace of oxygen it tended to elicit or to augment the large-waved "tone rhythm" already mentioned (Fig. 5 *D* and 5 *E*).

Histamine, also, was without effect in an atmosphere of nitrogen (Fig. 5 *F*).

Adrenaline, when oxygen was present, caused a rise in tone in the pregnant uterus of the cat, but had no effect in nitrogen (Fig. 2 *F*). The circular muscles of the mesenteric arteries, however, responded to adrenaline even in the presence of nitrogen, and this contraction persisted with practically unabated strength until admission of oxygen brought about a still further rise in tone followed by a slow decline, presumably due to oxidation of the drug (Fig. 4 *D*). These results with adrenaline are somewhat vitiated by the fact that, at that time, the nitrogen still contained a minute trace of oxygen.

As already mentioned it is conceivable that, in the absence of oxygen, tissues may be able to function, for a time at least, if there be present a hydrogen acceptor other than oxygen.

Lundberg(10) has found that methylene blue in very weak concentration acts as a stimulant to smooth muscle in the presence of oxygen.

In relatively strong concentration the effect is that of inhibition. The stimulant action is shown in Fig. 1 *C*. Methylene blue, however, was quite

Fig. 5. *A*. Rabbit bowel, effect of S—S glutathione in oxygen. Note stimulation, inability to prevent action of nitrogen on tone but the very marked persistence of fine rhythm. *B*. Virgin guinea-pig uterus, effect of S—S glutathione in oxygen. Note inability to prevent action of nitrogen on tone. *C*. Bitch uterus, effect of cystine in atmosphere of nitrogen. *D*. Virgin guinea-pig uterus, effect of pituitary extract in oxygen. Read right to left. *E*. Same preparation as in *D*, absence of effect of pituitary extract in nitrogen. Read right to left. *F*. Rabbit bowel, absence of effect of histamine in nitrogen. Read right to left. Time traces in minutes.

unable either to prevent the action of nitrogen or to raise the tone in the absence of oxygen. On theoretical grounds, this is not to be wondered at, for the hydrogen which a non-toxic dose of methylene blue can accept is equivalent to a very minute fraction of the oxygen requirements of even such a sluggish tissue as smooth muscle. The methylene blue showed no obvious sign of conversion to the leuco form.

Another possible hydrogen acceptor is oxidised, or S—S glutathione. Samples of this di-peptide were prepared from yeast and from skeletal muscle according to the instructions of Hopkins(3). Tsubura(14) found that smooth muscle is richer in glutathione than skeletal muscle, whose content in reduced glutathione, according to Tunnicliffe(15), is on the

average 0·04 p.c. to 0·045 p.c. Sufficient S—S glutathione was therefore added to give a total concentration in the bath of 0·05 p.c.

In oxygen S—S glutathione had a stimulant action on rabbit bowel and on guinea-pig uterus (Figs. 5 *A* and 5 *B*). In nitrogen no effect was observed with the guinea-pig uterus, but the rabbit bowel showed a restoration or definite increase in fine rhythmical movement (Fig. 6). In no case was the tone raised in nitrogen through the agency of glutathione, nor was the effect of oxygen on the general tone inhibited.

One cannot exclude, in the case of S—S glutathione, the possibility of a direct action on the preparation, a drug action not inhibited by oxygen lack, but it is perhaps significant that the amount of oxidised glutathione used was capable of accepting hydrogen equivalent to an amount of oxygen sufficient to supply the tissue for a prolonged period of time. No obvious nitro-prusside test, however, was ever detected in the contents of the bath.

Cystine, in the few cases in which it was tried, raised the tone even in nitrogen (Fig. 5 *C*). Cysteine and reduced S—H glutathione were without effect in oxygen or in nitrogen.

Fig. 6. Rabbit bowel, effect of oxygen lack and of S—S glutathione.

Discussion of results.

It might be objected that the nitrogen, after passing through the solution of alkaline $Na_2S_2O_4$, in spite of subsequent thorough washing, contained a toxic substance. Such results, however, were not confined to use of nitrogen alone, for, in a previous series, hydrogen, when substituted for oxygen, gave similar effects. The more sensitive tissues, too, reacted with nitrogen taken directly from the cylinder, in spite of its traces of oxygen.

The action of oxygen lack is also in keeping with the action of NaCN, which, although it does not completely abolish the consumption of oxygen, greatly retards it. Johnson, McClosky and Voegtlin, however, found that both oxygen lack and NaCN raised the tone in visceral muscle. There must be some undiscovered difference between their conditions and those under which the present work was carried out. Generalisations under the circumstances are inevitably insecure since there is no justification for the assumption that the various types of smooth muscle which have been employed resemble one another except in the grossest characteristics. Such generalisations as are made, then, must always be open to exceptions.

If the usual hypothesis for striated muscle contraction be accepted also for smooth muscle, the behaviour of the fine rhythmical movements in absence of oxygen is in harmony with such a conception. Here we have a period of relatively long duration in which the rhythmical contractions persist and finally diminish in size as the hypothetical store of energy-giving material is exhausted without restoration by means of the agency of oxygen. The action of glutathione lends support to this attitude.

The behaviour of the tonus of the preparation is not so easily explained. Lovatt Evans(8) has shown that the greater the tone of a preparation the less the actual contemporary consumption of oxygen, yet the tonicity is far more sensitive to complete oxygen lack than is the fine rhythm. The mere maintenance of a state of tone, also, probably does not entail an expenditure of energy at all comparable with that of a series of contractions, so that the rapid fall in tone can hardly be due to insufficiency of some direct energy-yielding oxidative process, nor due to exhaustion with failure of restoration in an anaerobic process such as we have in striated muscle.

As suggested by Lovatt Evans(9), the loss of tone might be explained as due to accumulation of end products which would normally be removed by oxidation, but this view presents several difficulties. If we

take lactic acid as a type, experiment shows that this accumulates very slowly in nitrogen and is only slowly removed on restoration of oxygen, yet the changes in tonus, especially the recovery on returning to oxygen, occupy only a few minutes. Moreover, as mentioned above, there is no correlation between the period of exposure to nitrogen and the rate of recovery of tone. This absence of correlation might of course be due to absence of further metabolic activity after a certain threshold duration in nitrogen, but again the rate of accumulation of lactic acid does not support this view.

One way out of the difficulty might be to look upon tone, as do Gross and Clark, as of neurogenic and the rhythmical movements as of myogenic origin. This postulates that such nervous tissue as is present in excised smooth muscle is more sensitive to oxygen lack than is the muscle substance itself. We are then faced with the persistence of coordinated peristaltic movement in preparations suspended in Trendelenburg's apparatus, which movements are supposed to depend on the functioning of the nerve net in the bowel. Actually, in this apparatus, fall in tonicity seemed to permit of freer expression of the ordinary contraction process (Fig. 3 *B*). Trendelenburg's apparatus, however, as mentioned above, did not allow of a wholly satisfactory attainment of oxygen lack.

The experiments indicate clearly that tonus, which is a characteristic property of the living plain muscle cell, is rapidly but reversibly suppressed when oxygen is withheld. That is to say, the physical state we know as tonus is a labile state, which we can best regard as the result of a dynamic equilibrium in the chemical aspect of which oxygen plays an important part. The similarity between the effects of withdrawal of oxygen and addition of cyanide seems to leave no alternative but to suppose that the processes concerned are oxidative in nature. In their absence tonus disappears altogether; the occurrence of oxidation is not the only factor concerned in tonus, but it is a *sine qua non*. In the absence of oxygen, moreover, as experiments by Miss A. Winner in this laboratory have shown, the tissue dies much sooner than it otherwise would.

Recent investigations by A. V. Hill(17) have suggested that in striated muscle under anaerobic conditions disintegrative changes occur owing to the failure of those oxidative processes which are essential for the maintenance of the living structure, and it would seem that the phenomena described in the present paper are capable of a similar interpretation.

It is not suggested here that the maintenance of the tonic state involves in itself the expenditure of energy, but merely that a certain amount of oxidation is necessary in order to keep the cell structures in

such a condition that tonus may occur. Indeed, after prolonged oxygen lack not only is tonus abolished, but so also is the property of contractility: failure to contract however is of later onset. Like the capacity for tonus, it is restored quickly when oxygen is re-admitted.

Summary.

(1) Lack of oxygen causes, in the great majority of cases, a rapid fall in tone of excised surviving visceral muscle.

(2) The fine rhythmical movements exhibited by smooth muscle persist for a time in the absence of oxygen, but they also finally disappear.

(3) Certain drugs, active in oxygen, fail to act in its absence.

(4) Oxidised glutathione partly restores rhythmical movement, but has no effect on tone.

(5) Tone seems to be a manifestation of a labile activity different from that producing rhythmical movement, and it is suggested that an essential condition for its appearance is the occurrence of oxidative reactions whereby the physico-chemical integrity of cell structures is maintained.

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THE CHOLESTEROL CONTENT OF NORMAL TISSUES AND THE EFFECT OF INTRAVENOUS INJECTIONS OF CHOLESTEROL THEREON.

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For convenience this paper will be divided into two sections, the first dealing with the cholesterol content of normal tissues in the rabbit, and the second with such modifications as were produced by intravenous injections of cholesterol.

The first section was undertaken with the object of obtaining such light on the physiology of cholesterol as could be derived from a rough comparison of the cholesterol content of different tissues, and also of providing adequate controls to the experiments in Section II. The second section was expected to give information on problems of storage and metabolism, and the part taken by different organs in these processes.

Section I. The cholesterol content of normal tissues.

Many estimations of blood cholesterol have been made, but the estimations of cholesterol in other tissues available in the literature are still very few, especially in the case of the suprarenals and the spleen. As the writer's work has been principally concerned with these organs, most attention has been paid to them in the present investigation, though estimations have also been made in the liver, brain, muscle, kidneys, lungs, and blood, for comparison with those of other workers.

Experimental. Method of estimation.

It was impossible to use the digitonin method of estimation owing to the expense of this substance, and the large number of experiments made. The method adopted, therefore, was that described by Grigaut(1). This consists in boiling the weighed tissue in a flask containing 70 p.c. alcohol, and 1 p.c. sodium hydrate until the tissue is dissolved, and is about 15 c.c. in volume. It may be mentioned here that in the case of some tissues, notably the liver, complete solution cannot be obtained, though the semi-solid suspension is more or less uniformly distributed through the solution. These 15 c.c. are then put into a separating funnel graduated to 15

and 30 c.c., cooled under running water, and then extracted twice with ether. The ethereal extract is washed each time with distilled water, and then evaporated to dryness. The residue is dissolved in chloroform and compared colorimetrically with a standard solution of cholesterol. The colour reaction used was that of Liebermann and Burchard, which consists in the addition of 2 c.c. of acetic anhydride, and 0·2 c.c. of pure sulphuric acid to the chloroform extract of cholesterol, and the subsequent development of a green colour. It was found essential to the proper development of this colour that all reagents and containers should be quite free from water, and that a definite fixed amount of sulphuric acid should be used in all cases, and not a "few drops" as sometimes advocated.

A number of duplicate estimations were made to test the adequacy of the results for the present purposes. When the samples were taken from the same finely minced organ the results never differed by more than 10 p.c. With samples not previously mixed by mincing, it was found that the results might differ as much as 18 p.c., doubtless because samples taken from different parts of an organ are not identical. This was particularly the case with the liver.

Owing to these discrepancies in certain of the estimations it should be mentioned that in comparing the results in Section II with the controls of Section I, no variation of less than 20 p.c. has been accounted of any significance. This rule allows a wide margin for experimental error, for it will be seen that the differences upon which any arguments were based, were at least 40 p.c., and sometimes as much as 300 p.c. Where duplicate observations have been made the average between the two results has always been used. In the case of the suprarenals and spleen the whole organ was used in the estimations to avoid errors of sampling.

All results are based on the wet weight of the tissue.

Results.

The results are given in tabular form. The findings are expressed in "milligrams per cent." (*i.e.* per 100 grm. of fresh tissue), except in the case of the suprarenals and brain where grams per cent. is more convenient. In addition to the percentage, the total amount of cholesterol is given for those tissues where it was possible to use the whole organ for the estimation.

TABLE I.

Cholesterol content of normal tissues.

		Suprarenals.				
Rabbit No.	Weight in grm.	Weight of adrenals in grm.	Grm. of adrenal per kilo body weight	Average percentage cholesterol (grm.)	Total cholesterol (mg.)	Total cholesterol per kilo body weight (mg.)
35	1320	0·480	0·363	6·0	29	22
45	1520	0·226	0·148	11·1	25	16
29	1640	0·210	0·127	6·3	13	8
40	1710	0·570	0·333	10·7	61	36
38	1800	0·330	0·183	6·0	20	11
44	1860	0·452	0·243	6·6	30	16
30	2500	0·330	0·132	10·0	33	13
33	1970	0·420	0·213	6·8	29	14
48	1370	0·230	0·167	7·8	18	13
54	1890	0·322	0·170	5·0	16	8
57	1700	0·498	0·293	4·1	20	12
73	1810	0·380	0·209	6·0	23	12
75	1930	0·500	0·259	—	—	—
85	1440	0·160	0·111	5·7	9	6
86	1500	0·270	0·180	11·1	30	20
95	1670	0·350	0·210	3·9	14	8
96	1740	0·595	0·342	—	—	—
122	1800	0·480	0·270	8·4	40	23
131	2080	0·420	0·202	9·9	41	24
143	1820	0·430	0·230	7·0	30	16
		Average	0·219	7·3	27	15

TABLE II.

Cholesterol content of normal tissues.

		Spleen.				
Rabbit No.	Weight in grm.	Weight of spleen in grm.	Grm. of spleen per kilo body weight	Cholesterol (mg. p.c.)	Total cholesterol (mg.)	Total cholesterol per kilo body weight (mg.)
65	—	—	—	430	—	—
68	—	0·510	—	470	—	—
70	—	—	—	400	—	—
73	1810	0·477	0·263	370	1·7	1·0
75	1930	0·510	0·264	270	1·4	0·7
85	1440	0·360	0·250	350	1·2	0·8
86	1500	0·515	0·345	350	1·8	1·2
87	1600	0·640	0·400	350	2·2	1·4
89	1300	0·715	0·550	260	1·9	1·4
92	2350	0·550	0·234	370	2·0	0·8
95	1670	0·300	0·180	420	1·2	0·7
97	1750	0·620	0·354	290	1·8	1·0
131	2080	0·650	0·310	480	3·1	1·5
133	1900	0·760	0·400	390	2·9	1·6
143	1820	0·610	0·330	520	3·2	1·7
		Average	0·281	380	2·0	1·15

TABLE III.

Cholesterol content of normal tissues.

Rabbit No.	Weight in grm.	Liver (mg. p.c.)	Muscle (mg. p.c.)	Brain (grm. p.c.)	Lung (mg. p.c.)	Kidney (mg. p.c.)	Blood (mg. p.c.)
33	1970	220	—	—	—	—	—
35	1320	350	—	—	—	—	—
40	1710	350	—	—	—	—	76
44	1860	250	—	—	—	—	79
45	1520	180	—	—	—	—	72
48	1370	270	—	—	—	—	69
54	1890	310	—	—	—	—	94
57	1700	450	—	—	—	—	79
73	1810	220	—	—	310	300	—
75	1930	—	64	—	290	320	—
85	1440	—	55	—	290	520	—
86	1500	290	68	—	490	460	—
92	2350	—	56	—	500	510	—
95	1670	—	77	1·8	—	510	—
96	1740	250	—	1·5	—	—	—
122	1800	—	—	1·9	—	—	—
131	2080	—	—	1·7	—	—	—
143	1820	—	—	2·2	—	—	—
	Average	285	64	1·8	376	436	78

DISCUSSION.

Varying with their richness in cholesterol the tissues examined fall into three groups. Firstly, the brain and suprarenals, which contain respectively an average percentage of 1·8 and 7·3 grm., and are thus the richest in cholesterol. Secondly, the spleen, liver, kidneys, and lungs, which do not differ materially in their content, containing an average of between 300 and 500 mg. p.c. in the majority of cases. Lastly, blood and muscle may be classed together as being the poorest in cholesterol, the blood containing an average of 78 mg. p.c. and the muscles an average of 64 mg. p.c.

Suprarenals. From the tables one striking fact stands out, namely that compared with other tissues the suprarenals contain a very variable amount of cholesterol. The percentage varies roughly from 4 to 11 grm. whilst the total amount in the glands varies from 9 to 60 mg. This probably indicates that the glands take an active part in cholesterol physiology, the amount found in the glands reflecting varied physiological states.

The suprarenals contrast forcibly with the brain in this respect, as the brain, though also containing a large amount of cholesterol, shows only small variations. The variations in other tissues are not sufficiently great to give any clue as to whether they take an active part in cholesterol physiology.

The cholesterol of whole blood was estimated in most cases by Leiboff's method (2), which, like Grigaut's method for the tissues, gives values for the total cholesterol. A few estimations were also made on the blood serum by Grigaut's method. Four such estimations gave an average of 37 mg. p.c., about half the value usually obtained in rabbits for whole blood. This is an interesting contrast with human blood where the amounts in serum and whole blood are approximately equal.

Results of other authors. The results given here for brain, liver, kidneys, lungs, and blood, agree moderately well with those recorded by Ellis and Gardner (3) who used the more accurate digitonin method of estimation, and also with those given by Baumann and Holly (4) for kidney.

The few estimations made by Landau and McNee (5) for the suprarenals led them to regard 10 p.c. as an abnormally high value. The present larger series of observations show that 10 p.c. is by no means uncommon, and must be regarded as falling within physiological limits.

Since completion of this work Dr E. J. Baumann of New York has kindly allowed me to compare some of his unpublished figures for the cholesterol content of the suprarenals with my own. The method of estimation was similar, and the results are in close agreement.

Section II. The effects of intravenous injections of cholesterol.

The effect of feeding cholesterol to animals has been investigated by numerous workers (Landau and McNee (5); Rothschild (6); Sternberg (7); Zinserling (8)), who, however, have paid most attention to the histological changes produced, and have made very few chemical estimations. The injection of cholesterol directly into the blood stream has been attempted only on a few occasions, and whilst the histological changes produced are described in some detail no chemical analyses are given. Dewey (9) records experiments of this nature, and notes an infiltration of the liver with cholesterol, a variable infiltration of the suprarenals, but no apparent increase in the spleen. Since, however, the staining reactions of a substance such as cholesterol can be greatly modified by its association or combination with other substances in the tissues, histological methods are of little quantitative value.

The objects of the present work, therefore, were to ascertain by chemical estimations what gross modifications could be produced in the cholesterol content of various organs by intravenous injections of this

substance. It was hoped that the results would throw light on certain aspects of the physiology of cholesterol.

Experimental.

It was found difficult to prepare emulsions of cholesterol which were sufficiently perfect to pass through the capillaries without untoward results though numerous methods were tried. Those which were unsatisfactory, as judged by the size of the particles under the microscope, and by the fact that injected animals died, need not be mentioned here. The following method, however, usually gave good results:

0·464 grm. of oleic acid and 0·75 grm. of cholesterol were weighed into the same test-tube and acetone was added in sufficient quantities (about 10 c.c.) to dissolve the whole when heated. The acetone solution was then poured little by little into a measuring cylinder containing 16·4 c.c. *N*/10 NaOH, *i.e.* the amount required to neutralise the oleic acid. The vessel was constantly agitated during the operation. A fine emulsion resulted which was concentrated by heating over a water bath until the volume was about 18 c.c., so that the emulsion contained approximately 4 p.c. of cholesterol.

The emulsion was used in amounts of 2 to 7 c.c. and injected into the veins of the ears very slowly. In the majority of cases there were no ill effects, though occasionally the animal showed signs of collapse for a few minutes, and then recovered.

If the emulsions were kept longer than 14 days they seemed to deteriorate, and it is advisable that they should be made up fresh in small quantities as required.

RESULTS.

The effects of the cholesterol injections will be seen in the various tables. They may be discussed best under the headings of the different tissues.

Blood. The blood cholesterol was estimated in numerous cases just before the animal was killed. The blood was taken from the opposite ear to the one used for cholesterol injection. The cholesterol content was raised in all cases except one, but not to such a marked degree as was anticipated. The average increase was about 50 p.c. These findings accord with those of Dewey (9), but are opposed to those of Weltmann and Biach (10), who stated that a substance such as cholesterol which gains access to rabbit serum cannot be readily eliminated.

The rapidity with which cholesterol is removed from the blood, however, is variable, for in one case (Rabbit 109) the content was normal

3½ hours after the injection, whilst in another (Rabbit 84) the cholesterol was still raised to 151 mg. p.c. (normal 78 mg. p.c.) 48 hours after the injection.

TABLE IV.

The effects of intravenous injections of cholesterol.

Rabbit No.	Weight in grm.	Milligrams of cholesterol injected	Period between injection and death (hours)	Blood cholesterol (mg. p.c.)	Liver cholesterol (mg. p.c.)	Lung cholesterol (mg. p.c.)	Kidney cholesterol (mg. p.c.)	Muscle cholesterol (mg. p.c.)	Brain cholesterol (grm. p.c.)
64	2060	190	4½	—	220	—	—	—	—
67	1700	250	3	151	250	—	—	—	—
69	—	170	6½	95	370	—	—	—	—
72	1700	190	6	101	210	550	360	—	—
74	1620	115	18½	136	430	420	490	—	—
80	1120	160	19	173	410	510	340	—	—
82	1760	200	24	139	470	150	470	—	—
84	1220	180	48	151	230	470	390	—	—
90	1700	180	24	105	430	600	440	—	—
91	2720	200	17½	117	—	250	—	67	1·8
94	2000	150	4	—	—	—	480	45	2·0
102	1950	250	1½	—	660	450	390	—	—
106	2500	250	24	—	370	—	—	—	—
109	1150	260	3½	78	420	—	—	—	—
128	1810	180	18½	—	—	—	—	—	—
			Average	124	372	425	420	56	1·9

TABLE V.

The effects of intravenous injections of cholesterol on the spleen.

Rabbit No.	Weight in grm.	Weight of spleen in grm.	Grm. of spleen per kilo body weight	Cholesterol (mg. p.c.)	Total cholesterol (mg.)	Total cholesterol per kilo body weight (mg.)
64	2060	—	—	600	—	—
67	1700	—	—	1040	—	—
69	—	—	—	1050	—	—
72	1700	0·757	0·445	1020	7·7	4·5
74	1620	0·602	0·372	740	4·4	2·7
80	1120	0·815	0·727	710	5·8	5·2
82	1760	0·700	0·397	780	5·4	3·1
84	1220	0·825	0·655	380	3·1	2·5
90	1700	1·470	0·865	610	8·9	5·2
91	2720	1·025	0·376	870	8·9	3·3
94	2000	0·645	0·322	880	5·7	2·8
102	1950	0·250	0·128	1340	3·3	1·7
106	2500	0·700	0·280	470	3·3	1·3
109	1150	0·340	0·290	490	1·7	1·4
			Average 0·441	780	4·9	2·8

TABLE VI.

The effects of intravenous injections of cholesterol on the suprarenals.

Rabbit No.	Weight in grm.	Weight of suprarenals in grm.	Grm. of adrenal per kilo body weight	Cholesterol (grm. p.c.)	Total cholesterol (mg.)	Total cholesterol per kilo body weight (mg.)
64	2060	0·540	0·262	4·9	—	—
67	1700	0·200	0·118	1·9	—	—
69	—	0·247	—	3·4	8·4	—
72	1700	0·322	0·189	6·8	21·9	12·9
74	1620	0·217	0·134	5·0	10·9	6·7
80	1120	0·165	0·147	4·5	7·4	6·6
82	1760	0·408	0·230	3·6	14·7	8·4
84	1220	0·275	0·230	5·4	14·8	12·4
90	1700	0·520	0·310	5·0	26·0	15·3
91	2720	0·460	0·170	5·1	23·4	8·6
94	2000	0·330	0·165	4·0	13·1	6·6
102	1950	0·380	0·195	6·5	24·6	12·6
106	2500	0·370	0·148	4·2	15·4	6·1
109	1150	0·340	0·290	5·2	17·6	15·0
128	1810	0·330	0·180	4·3	14·3	7·9
		Average	0·196	4·6	17·7	9·9

Suprarenals. The German school supposes that the suprarenals are concerned in storing cholesterol, and that the amount present in the glands is proportionate to the amount in the blood. If this were so hyper-cholesterinæmia would be expected to result in an increased cholesterol concentration in the suprarenals. In the present experiments, however, no such increase was found. On the contrary there was a definite decrease. The average percentage decreased from 7·3 in normal animals to 4·6 in cholesterol injected animals. Also the total amount of cholesterol in the glands was correspondingly diminished. This was not due to any variation in the weight of the suprarenals, since their average weight proportionate to the body weight remained constant. It is possible that part of the decrease was due to the sodium oleate with which the cholesterol was administered since injections of this substance alone gave a similar though much smaller decrease, but it is improbable that the whole could have been due to the sodium oleate.

The French authors, contrary to the German, regard the suprarenals as mainly concerned in the synthesis of cholesterol, and as it has been shown (Fraser and Gardner, Nomura, Sano, and others (11, 12, 13)) that the body may also derive cholesterol from food, it seems reasonable to suppose that a balance is normally maintained between the exogenous and endogenous supply, so that an increase in exogenous cholesterol might be expected to produce a diminished endogenous production.

If such a theory were correct the decrease in the cholesterol of the

suprarenals in the present experiments might be interpreted as indicating a diminished production on the part of the glands due to an increase in exogenous supply following the intravenous injection.

Spleen. Of all the tissues examined this organ showed the greatest changes. The percentage of cholesterol was doubled, and, as at the same time the average weight of the organ was considerably increased, the total amount of cholesterol in the spleen was three or four times its normal value. It is evident, therefore, that, weight for weight, the power of removing excess cholesterol from the blood is greater in the spleen than in any other tissue.

The question arises as to whether this property is exercised by the spleen in the normal animal. It may be suggested that by use of this function the spleen may normally regulate the level of the blood cholesterol, continually removing small excesses. In support of this theory it is to be noted that most authors (Eppinger, King, Sotti and Torri(14, 15, 16)) find that removal of the spleen produces hyper-cholesterinæmia, though Bodansky(17) found no such increase in blood cholesterol, nor did Randles and Knudson(18) after splenectomy in rats. In criticism of the theory it may be urged that in the experiments described here, the spleen merely removes cholesterol as it would remove any other foreign particulate substance, for example, silver or Indian ink, from the blood stream.

It should be pointed out, however, that even in cases of hyper-cholesterinæmia where there is reason to believe that the cholesterol is in a similar form to that in which it normally circulates, a great excess is found in the spleen. This is illustrated by a case reported recently by Dyke(19) where hyper-cholesterinæmia of 1250 mg. p.c. was present, associated with great enlargement of the spleen which contained 4½ times the normal percentage of cholesterol.

Liver. The liver also showed an increase in its cholesterol content following intravenous injections, but this was very much smaller than in the case of the spleen. Nevertheless it is evident that the removal of small amounts by such a large organ as the liver would be equivalent to the removal of a very considerable total amount. It is possible that the liver may also take a part in maintaining the blood cholesterol at a constant level.

Brain, kidneys, lungs, and muscle. None of these tissues showed any material change in its average cholesterol content after intravenous injection.

The effect of repeated injections of cholesterol.

Four experiments were made in which in place of the usual single dose of cholesterol two to four injections were made extending over a period of two to four days.

The results were similar to those obtained by single injections, the spleen showing the most marked increase in cholesterol, and the liver a lesser increase. One important difference, however, will be observed. Instead of the customary decrease in the cholesterol content of the suprarenals the average value is almost identical with that of normal buck rabbits. Evidently the influence exerted by the cholesterol injections on the suprarenals is not sustained if the injections are repeated.

TABLE VII.

Effects of repeated injections of cholesterol.

Rabbit No.	Weight in grm.	Cholesterol injected (mg.)	Period over which given	Period since last dose	Suprarenals cholesterol (grm. p.c.)	Liver cholesterol (mg. p.c.)	Spleen cholesterol (mg. p.c.)	Kidney cholesterol (mg. p.c.)
88	1700	210	48 hours	24 hours	—	—	920	350
113	1680	650	3 days	24 hours	9·5	470	910	530
115	1390	385	48 hours	3 days	5·4	280	750	220
117	1880	785	4 days	23 hours	7·7	580	690	390
				Average	7·5	440	820	370

Effects of reticulo-endothelial blockade.

On histological grounds it has been suggested by Rothschild(20), Zinserling(8), McMeans(21), and Khalatov(22) that cholesterol can be taken up by the Kupfer cells of the liver, and it seemed probable in the present experiments that the reticulo-endothelial elements of both the spleen and the liver might be responsible for the removal of the excess of cholesterol. If this were the case, a blockade of the R.E. system, followed by intravenous injections of cholesterol, would be expected to interfere with the removal of cholesterol by these organs.

Experiments were, therefore, made in which the R.E. system was blocked by intravenous injections of Indian ink for several days, followed by the usual injection of cholesterol. Bedson(23), in his work on blood platelets, found blockade of this description to be effective.

It will be seen from the tables, however, that the blockade did not prevent the accumulation of cholesterol in the spleen, though the degree of infiltration was not quite so marked as usual. In the case of the liver the degree of cholesterol infiltration was not materially altered.

TABLE VIII.

Effect of sodium oleate injections on tissue cholesterol content.

Rabbit No.	Weight in grm.	Sodium oleate solution injected	Supra-renals chole-sterol (grm. p.c.)	Total chole-sterol in supra-renals (mg.)	Total chole-sterol per kilo body weight (mg.)	Spleen chole-sterol (mg. p.c.)	Liver chole-sterol (mg. p.c.)	Kidney chole-sterol (mg. p.c.)	Brain chole-sterol (grm. p.c.)
110	1450	4·5 c.c. 1·6 p.c.	4·1	9	6	340	290	—	—
112	1400	4·5 c.c. 5 p.c.	10·6	16	11	440	280	—	1·7
116	2400	5·5 c.c. 2·5 p.c.	7·6	31	13	410	—	490	—
119	2120	6·5 c.c. 2 p.c.	3·7	16	7	420	—	470	1·6
148	2085	6 c.c. 2 p.c.	3·9	34	16	480	510	—	—
150	1820	6 c.c. 2 p.c.	6·0	33	18	370	440	—	2·0
		Average	6·0	23	12	410	380	480	1·8

TABLE IX.

Effect of Indian ink injections on tissue cholesterol content.

Rabbit No.	Grm. of Indian ink injected	Period of action	Blood chole-sterol (mg. p.c.)	Liver chole-sterol (mg. p.c.)	Spleen chole-sterol (mg. p.c.)	Lung chole-sterol (mg. p.c.)	Supra-renals chole-sterol (grm. p.c.)
103	0·210	3 days	85	350	370	440	4·8
104	0·087	18½ hours	—	370	450	—	8·1
120	0·385	3 days	—	370	570	—	8·4
		Average	360	460	—		7·1

Effect of blockade of the R.E. system with Indian ink followed by injection of cholesterol.

Rabbit No.	Grm. of Indian ink injected	Period of action	Amount of chole-sterol injected (mg.)	Period of action (hours)	Liver chole-sterol (mg. p.c.)	Spleen chole-sterol (mg. p.c.)	Supra-renals chole-sterol (grm. p.c.)	Kidney chole-sterol (mg. p.c.)
107	0·140	18 hours	187	18½	470	600	3·0	380
108	0·385	2 days	265	19	570	660	2·5	280
129	0·420	3 days	200	6¾	440	790	5·3	480
				Average	490	680	3·6	380

The experiments cannot be regarded as conclusive and are capable of two interpretations, either that the power of the spleen and liver to remove excess of cholesterol does not depend on their reticulo-endothelial

elements, or alternatively that the blockade was not complete. The latter explanation seems the more probable, especially in the light of previous histological work, and of the slight decrease in the amount of cholesterol removed by the spleen after blockade.

Summary.

1. Tables are given showing the cholesterol content of the suprarenals, spleen, brain, liver, kidneys, lungs, muscle, and blood in normal buck rabbits.

2. The great variations in the amount of cholesterol in the suprarenals as compared with other tissues suggest that these organs take an active part in cholesterol physiology.

3. Intravenous injections of cholesterol resulted in:

(*a*) A definite hyper-cholesterinæmia, though less than would have been expected, showing that the cholesterol has been removed rapidly from the circulation.

(*b*) The *suprarenals* showed no increase in cholesterol, but on the contrary a definite decrease.

This, it is argued, is in opposition to the idea of storage in these organs, and indirectly supports the theory of synthesis.

(*c*) The *spleen* showed a marked increase in its percentage and total amount of cholesterol, removing proportionate to its weight a greater amount of cholesterol than any other tissue.

It is suggested that the spleen may be concerned normally in maintaining the blood cholesterol at a fixed value by continually removing small excesses.

(*d*) The *liver* takes up a smaller amount of cholesterol as judged by the percentage of cholesterol present. It may also normally have the function of maintaining the blood cholesterol constant.

(*e*) The effects of reticulo-endothelial blockade did not decide the question as to whether the power of the spleen and liver to remove cholesterol depends on their reticulo-endothelial elements.

(*f*) The *lungs, kidneys, brain, and muscle*, showed no appreciable change after intravenous injection of cholesterol.

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THE MEASUREMENT OF THE OXYGEN CAPACITY OF HÆMOGLOBIN.

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Within recent years a great number of determinations (which need not be mentioned in detail) have given the impression that the ferricyanide method of oxygen estimation has yielded lower results for the oxygen capacity of blood than the Van Slyke pump. This difference existed between the Van Slyke pump and both forms of ferricyanide apparatus as currently used—Haldane's constant pressure model and Barcroft's differential model. Such a difference has been explained: (1) on the assumption that reducing substances in the plasma, as shown by Douglas(1), Parsons and Parsons(2), and also by Litarczek(3), use up oxygen during the time in which the blood is being equilibrated in the apparatus; (2) that reducing substances in the corpuscles do the same, Morawitz and Röhmer(4), Douglas(5); (3) that the corpuscles are incompletely laked, Haldane(6), the unlaked portions not yielding their oxygen and (4) the blood may be contaminated with organisms, Haldane(6), which have an appreciable respiration.

All these explanations possess an element of truth, the first two certainly apply to blood of anæmic individuals and probably of rabbits. Harrop(7) has drawn a general relation between the rate of oxidation in blood and the number of reticulated red cells. Nevertheless they do not cover the whole ground, because, although they are not applicable to fresh hæmoglobin solutions (as opposed to blood), such hæmoglobin solutions on occasions exhibit the phenomenon which it is sought to explain. Thus:

Ox hæmoglobin.

Oxygen capacity in c.c. per 100 c.c. of hæmoglobin solution.

Solution	Haldane's apparatus	Van Slyke apparatus
I	16·20 (5)	16·96 (2)
II	17·28 (6)	18·59 (2)
III	18·27 (5)	19·42 (3)
IV	17·72 (9)	18·35 (2)
V	17·93 (4)	19·59 (3)

The figure given is in each case the average of a number of determinations, the number being stated in brackets. Thus for solution I the Haldane's apparatus(5) gave 16·42, 16·42, 15·91, 16·35, 15·91, while the Van Slyke pump gave 17·15 and 16·77.

Similar figures might be given for the differential method (9) as compared with the Van Slyke method. The phenomenon is, however, more difficult to investigate because the disparity is not constant; on occasions good agreement has been obtained. Thus:

Sheep's hæmoglobin.

C.c. Oxygen per 100 c.c. solution.

Solution	Differential method	Haldane's method	Van Slyke method
IV	14·35 (2)	14·70 (4)	14·90 (2)
V	17·81 (3)	17·77 (2)	18·06 (4)
VI	19·96 (3)	—	19·59 (4)

There are no cases of the differential or Haldane's method giving results seriously higher than the Van Slyke method. Therefore the discrepancy varies from nothing to about 5 p.c. of the gas given off. It is not due to the fact of using ferricyanide, for of course ferricyanide is used in the Van Slyke method.

The Van Slyke method differs from the others in two matters of principle: (1) the gas is given off to a vacuum so that there is only such partial pressure of oxygen in the atmosphere as is caused by the evolved gas; (2) the gas is given off on the instant of the blood entering the apparatus; so that no time is given for slow oxidations in the hæmoglobin solution or blood to vitiate the result. It seemed desirable to compare the three methods already mentioned with the old blood gas pump in which much larger quantities of hæmoglobin solution could be dealt with. In this way information might be obtained as to which method was correct and whether the defects of one or other were due to the smallness of the quantities of blood and gas which were dealt with. The present paper gives the results of a series of comparisons between the blood gas pump and the Van Slyke constant volume apparatus (10).

Apparatus. The pump used was so nearly like that described by Barcroft and Roberts (11) that it is unnecessary to do more than indicate differences in detail from the apparatus shown in Fig. 2 of their paper.

(1) Instead of the measuring burette shown (except in the determination of May 5, where 50 c.c. of hæmoglobin solution were used) a bulb of about 10 c.c. was used, the volume of which was known to about 1 part in 1000.

(2) A tap was fitted leading to the external air between the drying chamber and the condenser, the right angle bend in the figure becoming a T.

(3) The extracted gas was not received from the pump into a Bohr's

receiver and in it transferred to the blood gas apparatus, but into a special receiver which was blown on to the gas burette of the Haldane's gas

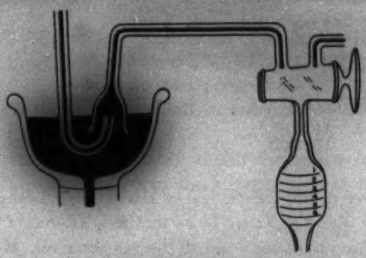

Fig. 1. *A* = end of "fall-tube" of pump. *B* = gas burette of Haldane's gas analysis apparatus.

analysis apparatus as shown in the figure—an arrangement which worked successfully. The extracted gas was drawn into the analysis apparatus without any danger of contamination with such air as lurks in rubber connections, moreover it was easy with this method to be sure that the pump was completely exhausted both before and after the extraction. Usually we regarded it as being vacuous if on three successive exhaustions no visible bubble of gas appeared in the inverted cap.

It is not to be supposed that on any occasion we had the sort of vacuum which would be required to satisfy the physicist who concerns himself with high vacua. Always after 24 hours' standing a few hundredths of a cubic centimetre of gas could be extracted from the apparatus and after a week's standing the gas was to be measured in tenths of a cubic centimetre. We did not investigate the nature of this gas very closely. It was not air (for a considerable portion could be absorbed by alkali) and therefore not due to leakage into the apparatus. We believe it came either out of the sulphuric acid in the drying chamber or out of the substance of the rubber corks, perhaps in part off the surface of the glass.

The nitrogen results, which are given below, speak for themselves. That of May 5 is of special interest as the amount of solution analysed was five times as great as in other cases with the pump. The error due to adventitious nitrogen should therefore have only been, in this case, one-fifth of what it was in others, yet they agree. These results confirm the findings of other workers in making the nitrogen higher than is found in the amount of distilled water present in 1 c.c. of hæmoglobin solution at any given temperature.

In the receiver of the pump we usually had about 10 c.c. of distilled water at the commencement of the experiment, this was warm.

Two series of determinations were carried out: (1) in which ferricyanide as well as water was put in the receiver to start with, (2) in which water only was put in the receiver. The results are given below. No analyses performed within the dates given have been omitted. They demand little comment.

Total O_2 and N_2 in volumes % obtained with the gas pump and with the Van Slyke apparatus from Hb solution saturated with air at room temperature.

Date of experiment. Species of Hb	Gas pump				Van Slyke apparatus			
	With ferricyanide		No ferricyanide		By Harrison		By Sendroy	
	O_2	N_2	O_2	N_2	O_2	N_2	O_2	N_2
April 14	20·50	1·84	19·46	1·74	19·91	1·74	20·29	1·46
Ox	20·39	1·61	20·17	*3·30	20·25	—	20·26	1·48
	20·45	—	—	—	—	—	—	—
April 16	18·17	1·95	18·16	2·01	—	—	18·12	1·49
Sheep	18·27	1·57	*14·20	1·72	—	—	18·13	1·47
	18·29	1·85	—	—	—	—	—	—
April 18	18·11	1·54	—	—	—	—	17·86	1·47
Sheep	18·04	1·45	—	—	—	—	17·83	1·53
April 19	—	—	17·78	1·85	—	—	17·82	1·47
Sheep	—	—	17·82	1·54	—	—	17·79	1·44
May 2	20·33	1·39	—	—	20·10	1·56	—	—
Sheep	20·25	1·44	—	—	19·93	1·48	—	—
	20·76	1·60	—	—	20·17	—	—	—
	20·06	1·40	—	—	20·19	—	—	—
May 4	20·18	1·37	—	—	20·15	—	—	—
Sheep	20·42	1·25	—	—	20·44	—	—	—
May 5	19·71	1·50	—	—	20·07	1·49	—	—
Sheep	—	—	—	—	20·10	1·50	—	—
May 7	—	—	20·22	1·29	20·23	1·51	—	—
Sheep	—	—	*18·92	1·27	20·18	1·55	—	—
May 8	—	—	20·54	1·58	20·39	1·66	—	—
Sheep	—	—	20·52	1·65	20·16	1·67	—	—
	—	—	20·63	1·57	20·24	1·91	—	—
	—	—	—	—	20·45	1·98	—	—
May 9	—	—	*18·83	1·57	20·60	1·54	—	—
Sheep	—	—	20·23	1·66	20·48	1·63	—	—
	—	—	20·40	1·50	—	—	—	—
Averages for N_2	—	1·55	—	1·62	—	1·61	—	1·48

As regards series (1). At first we thought that the pump gave higher results than the Van Slyke apparatus by about 1 p.c. of the gas given off, but of this we were not able to convince ourselves. In series (2) clearly there is very close agreement between the pump and the Van Slyke apparatus, except in certain cases (3 oxygens and 1 nitrogen) which are marked in the table with asterisks. Of these the first two were of some

interest because on these occasions, and on these alone, the hæmoglobin became brownish on entering the receiver, and we believe the water into which it was received was at too high a temperature. This was most marked on April 16 for the determination which gave 14·20. The determination on May 9 (18·83) remains unexplained. Otherwise it is clear that whether or not ferricyanide be put in the receiver of the pump, the blood gas pump and the Van Slyke constant volume apparatus give results which are very close to one another both for the oxygen and the nitrogen in hæmoglobin solutions.

Conclusion.

The blood gas pump and the Van Slyke constant volume apparatus agree in the analyses, both for oxygen and nitrogen, which they give of hæmoglobin solutions.

We take this opportunity of thanking Dr Van Slyke for the personal interest which he has taken in the work and the Rockefeller Institute for presenting one of the two Van Slyke apparatus (that used by Sendroy). The remainder of the current expenses have been provided by the Medical Research Council to which body our thanks are also due.

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THE ACTION OF GLUCOSONE UPON DIFFERENT SPECIES OF ANIMALS.

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Introduction.

Glucosone or oxyglucose, when injected subcutaneously, has been shown by one of us (A.H.(1)) to bring about in normal mice a train of symptoms which are very similar to those occasioned by a large dose of insulin. These observations have been confirmed in a large number of experiments and are now extended to white rats, rabbits, guinea-pigs and cats.

Action upon mice.

A dose of 2·4 mg. per gramme body-weight is non-lethal, but productive in from five to seven minutes of symptoms which in their incidence and general course closely resemble those of insulin. Smaller doses show no effects, immediately or later. Larger doses produce symptoms in from three to five minutes, convulsions, collapse and coma with death in about ten minutes from the time of injection. Just as in the case of insulin administration considerable variations occur in different mice in the extent to which they show phenomena of irritation, stiffening of tail and convulsions, and those of drowsiness, loss of muscular tone and collapse. On the whole glucosone is rather more productive of the latter phenomena than is insulin, but occasionally convulsions predominate in the early stages and the appearance is indistinguishable from a typical insulin effect. The margin between the dose of glucosone which produces symptoms and a lethal dose is a small one, 0·2 mg. per gramme body-weight. The glucosone effect differs from the insulin effect in its rapidity of development, completeness of recovery from a non-lethal dose, absence of relapses and the maintenance of body temperature. Less pronounced differences are the more frequent absence of irritative phenomena and a tendency on the part of the mouse to show backward rotatory movements.

Action upon white rats.

A dose of 1·8 mg. per gramme body-weight injected subcutaneously is productive of symptoms, while 2 mg. per gramme body-weight is fatal. Intraperitoneal injection gives effects which in their incidence are more rapid. In one animal an intraperitoneal dose of 1·71 mg. per gramme body-weight caused death in 12 minutes. Symptoms appear in from five to seven minutes after injection. The animal shows inability to cling to its wire cage, its muscles become limp and a sprawling attitude is assumed. The eyes are protuberant, convulsions may occur and are sometimes severe, at other times there are continuous twitching movements of the muscles of the limbs and body and convulsive starts. Respirations are rapid and gasping. Intervals of complete relaxation of the muscles are seen, and with a fatal dose death may occur suddenly in a convulsive seizure or follow in coma.

The symptoms produced by glucosone in the rat are very similar to those resulting from insulin but are not identical. They differ in their more rapid onset, absence of loss of temperature and of relapses during recovery, and the shortness of the convulsive stage. On the other hand the appearance of the animal during the convulsive and comatose stages closely resembles that of the insulinised rat, and under the influence of a dose which is almost lethal the symptoms of the animal are almost identical with those which it shows in the recovery from insulin. In one rat (dosage 1·34 mg. glucosone per gramme body-weight) which was comatose the intraperitoneal injection of a large dose of glucose had no visible effect upon its condition for more than ten minutes, but recovery took place thereafter slowly with occasional convulsions and much somnolence.

Action upon rabbits.

A solution of glucosone was injected subcutaneously, intraperitoneally or intravenously. A large dose given intravenously was immediately fatal in one animal, and the effects of the glucosone appeared more rapidly by intraperitoneal than by subcutaneous injection. Doses of about 1·7 mg. per gramme body-weight were effective in producing symptoms by the intraperitoneal route, and 2·39 mg. per gramme body-weight proved fatal. Symptoms are seen about five minutes after the injection of a toxic but non-lethal dose. The animal sprawls and collapses when attempting to move. Convulsions occur especially when it is handled, and a very noticeable phenomenon in the rabbit is a tonic contraction of the dorsal muscles of the neck. A typical attitude is assumed,

the legs are limp and may be spread out laterally so that the animal lies in a prone position with its head acutely dorsiflexed and the mouth and nose pointing vertically upwards. This attitude persists for a time until interrupted by general convulsions and rolling movements. Respiration is very rapid and shallow, cries frequent, eyes protuberant and pupils dilated. Relaxation of the muscles follows, but the retraction of the head reappears frequently. The condition may pass into coma, the muscles are relaxed, the respirations slow and gasping and the corneal reflex may disappear. With a non-lethal dose the condition gradually improves, the corneal reflex reappears, respirations improve and the tonic contraction of the neck muscles may come back to some extent. The muscles regain their tone and the animal eventually assumes a sitting posture but remains limp and sleepy for some time. Complete recovery may be seen within two hours from the time of injection. There is no loss of body temperature and a rise of 1° C. has been observed. The reducing power of the blood shows no diminution, but no attempt has been made to differentiate between the reduction due to glucose and that due to glucosone.

The symptoms are very like those produced by insulin but differ in the predominance of retraction of the head. This phenomenon, however, occurs in rabbits under insulin followed by a large dose of glucose. During the recovery stage after insulin and glucose rabbits show convulsive seizures which appear to be identical in character with those resulting from glucosone, and the typical posture of the glucosone effect is seen.

Action upon guinea-pigs.

A dose of 2 mg. per gramme body-weight of glucosone injected intraperitoneally produced severe symptoms, while 2·95 mg. per gramme body-weight proved fatal.

Symptoms of weakness of muscles occur in five minutes after injection, the hind limbs give way and the animal walks with difficulty. In a short time the guinea-pig lies in a prone position or on its side if so placed, but retains the power of orientating its head. This is eventually lost. Paddling movements of the limbs are characteristic but there are no convulsions. There is no protuberance of the eyes, urine and fæces were generally passed, and the animals became comatose with complete loss of the corneal reflex. The animal which received 2·95 mg. glucosone per gramme body-weight died in coma within two hours of the time of injection. The animal which received the smaller dose recovered completely in the same time. During the recovery stage twitching of muscles was prominent but there were no convulsions.

The guinea-pig shows more distinct differences between the glucosone and the insulin effects than do the other animals examined. Under the influence of insulin there is also loss of muscle tone, but the neck muscles lose their tone at an earlier stage and the head of the guinea-pig rests on the table, its power of orientation not persisting so long as it does under glucosone. Convulsions too are prominent under insulin but not under glucosone. Paddling movements of the limbs are common to both conditions. The recovery stage in both is very much alike.

Action upon cats.

Young animals only were used of weights between 567 and 1020 grm. owing to the limited amount of glucosone available. A dose of 5 mg. per gramme body-weight was fatal, and 2·35 mg. per gramme body-weight gave severe effects but was not lethal. The large dose mentioned produced symptoms in five minutes, but with the non-lethal dose no change was noted in the behaviour of the animal for ten minutes, when it began to mew and showed a disinclination to walk. This is quickly followed by the legs giving way and the head droops until the nose rests upon the table. When roused the cat can stand for a short time but staggers and collapses. As the condition develops the muscles become limp, the mouth gapes, mewing and salivation are constant, respirations gradually increase in rate (240 per minute recorded in one), and urine and fæces may be passed. There is some exophthalmos and the pupils, at first constricted, become widely dilated. No definite convulsions occurred in any of the cats, but twitching of the muscles was common and a certain amount of spasm of the extensors of the limbs and dorsiflexion of the head were noted. Half an hour after injection the animal may be comatose, lying on its side with no movements but those of rapid and shallow respiration. Recovery from a non-lethal dose takes place rapidly, the respirations diminish in frequency, twitching of the limbs follows, the pupils contract and the cat assumes a sitting posture with drooping head and closed eyes. With a smaller dose (0·97 mg. per gramme body-weight) the symptoms were similar but less severe and complete recovery took place in 40 minutes after the injection.

A similar sequence of symptoms is seen in cats after the injection of a large dose of insulin, but convulsions usually occur and may be a prominent feature. One of the animals which recovered from glucosone was given insulin after an interval of some weeks and its symptoms appeared identical with those produced by glucosone with the exception of a slight convulsion and a comparatively slow recovery.

Discussion of results.

Glucosone has been shown to exert a toxic action upon mice, rats, rabbits, guinea-pigs and cats. The method of administration, intravenous, intraperitoneal or subcutaneous, does not alter the nature of the symptoms produced, but the rapidity of development and the intensity of the effect vary, intravenous injection being the most and subcutaneous the least effective in these respects.

Just as a toxic dose of insulin gives rise to what may be called "species symptoms" so does a toxic dose of glucosone; thus, for example, we have mewing and salivation in the cat, head retraction in the rabbit and paddling movements of the limbs in the guinea-pig. The amount of glucosone which is requisite to produce toxic symptoms is remarkably constant, being in the neighbourhood of from 1 to 2 mg. per gramme body-weight. A relatively small increase in the dose is fatal. Small doses of glucosone appear to be innocuous, and no bad effects have been noticed from the repeated use of non-lethal amounts when spread over a sufficient period of time.

The symptoms produced by glucosone in each species of animal show a strikingly close resemblance to those which follow the injection of insulin. Recovery of an animal from a toxic but non-lethal dose of glucosone is also attended by symptoms which are very like those seen in the recovery of the same animal from insulin followed by the administration of glucose.

The question naturally arises as to what connection, if any, exists between glucosone and insulin. One of us (A.H.(1)) has already put forward the suggestion that glucosone arises from the action of insulin on the blood glucose. According to this view the injection of a large dose of insulin would result in the gradual conversion of the glucose of the blood into glucosone, which would be formed at a rate in excess of that at which it is normally utilised and so exert a toxic effect. Simultaneously there would be a hypoglycæmia of gradually increasing severity, which would in itself probably not give rise to symptoms until the convulsive level is reached. Accordingly the injection of glucose or of adrenaline to raise the blood sugar level dispels in an insulinised animal the convulsive symptoms, but leaves it liable to relapse, if the insulin remains in excess, and still suffering from the toxic action of glucosone.

The experiments recorded in the present communication lend support to the above interpretation of the action of insulin. Thus there is no evidence in our experiments that glucosone produces a deficiency of

glucose in the blood, though this is not impossible because, although the reducing power of the blood is high during the glucosone effect, there are no means at present available of distinguishing between the reduction due to the glucosone injected and that due to the glucose actually present. There is, however, evidence against a hypoglycæmia being produced by glucosone in that, in the first place, the injection of glucose has no beneficial effect upon the condition induced by the latter, and secondly, in small animals at least, the glucosone effect is not attended by the rapid fall of body temperature which is characteristic of the insulin effect. Finally, the convulsive phenomena, more especially in the guinea-pig and cat, are much less prominent under glucosone than they are under insulin.

The only other particular in which a difference was recognised is the rapidity with which the effects of glucosone are manifested. Five minutes in mice, rats, rabbits and guinea-pigs, and ten minutes in cats, suffice to produce a distinct loss of tone of the skeletal muscles and death may occur immediately during the intravenous injection of a lethal dose. The development of symptoms after insulin injection, on the other hand, takes some time owing to the length of the period necessary for the production of a sufficient concentration of the toxic compound, which we have suggested is glucosone. For the same reason hypoglycæmic convulsions, as a rule, do not occur until sufficient time has elapsed for the reduction of the blood sugar to a low enough level, and it occasionally happens that the irritative phenomena of insulin manifested in convulsions are slight or even absent, and the condition passes rapidly into one of coma.

With regard to the toxic action of insulin apart from the hypoglycæmia, Fischler(3) has recently suggested that this effect is due to the production of an excess of methyl glyoxal—$CH_3.CO.CHO$, which compound has been shown by Sjollema and Seekles(4) to exert a toxic action when injected into rabbits. We have confirmed the toxic effect of methyl glyoxal, but the symptoms which develop from its injection are not so suggestive of those following insulin as are those produced by glucosone. Both methyl glyoxal and glucosone, when present in excess in the organism, exert a toxic action, though of a different nature. This does not preclude, but in our opinion rather heightens, the probability of their possessing physiological significance, and as methyl glyoxal has long been regarded as an intermediate, so also may glucosone be concerned in carbohydrate metabolism.

The transformation of glucose into glucosone only necessitates the removal of two atoms of hydrogen from the molecule: thus

$$\underset{\text{(glucose)}}{CHO.CHOH.(CHOH)_3.CH_2OH} + O = \underset{\text{(glucosone)}}{CHO.CO.(CHOH)_3.CH_2OH} + H_2O.$$

is a type of oxidation which is probably of common occurrence in animal tissues, whereas the first oxidation product of glucose usually obtained *in vitro*, namely gluconic acid:

$$\underset{\text{(glucose)}}{CHO.(CHOH)_4.CH_2OH} + O = \underset{\text{(gluconic acid)}}{COOH.(CHOH)_4.CH_2OH},$$

is a compound of no known physiological importance.

That such a slight alteration in the molecule, as indicated above, transforms the inactive glucose into a highly active material, glucosone, which reduces Fehling's solution in the cold and exerts so toxic an action upon the organism, is indeed remarkable. Whatever may be the exact significance of glucosone there can be no question as to its possessing striking chemical and physiological activities.

Summary.

The injection of glucosone produces in mice, white rats, rabbits, guinea-pigs and cats, a train of symptoms which closely resemble but are not identical with those occasioned by a large dose of insulin.

The amounts of glucosone requisite to produce symptoms and a lethal effect are recorded for each of the above species.

Reasons are given why glucosone may be regarded as an important intermediate substance in carbohydrate metabolism.

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A COMPARISON OF THE EFFECTS OF ADRENALINE AND PITUITRIN[1] ON THE PORTAL CIRCULATION.

By G. A. CLARK.

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The action of adrenaline on the portal circulation has been investigated by many workers (1, 2, 3, 4, 5), and it is generally accepted that the rise in portal pressure following the injection of this substance is a result of constriction of the intrahepatic venous radicles (1, 2, 4), particularly of the more muscular sublobular veins (4), causing obstruction to the outflow from the portal vein. Some observers, however, have insisted that the rise in pressure is due to swelling of the liver cells, which thus compress the venous channels (5). That such swelling does occur in the intact animal as a result of adrenaline injection has been confirmed, but its effect on portal pressure is not of primary importance, as will be shown below; in fact, it seems probable that the swelling is secondary to venous constriction. If, then, adrenaline constricts the intrahepatic venous radicles it seemed that pituitrin (or its vasoconstrictor principle vasopressin), which appears to act on the smooth musculature of blood vessels (6), should produce a similar effect. But pituitrin has been shown to lower pressure in the portal vein (5), and it was to determine the significance of this phenomenon that the present investigation was undertaken.

Cats anæsthetised with amytal were used. Arterial blood-pressure was recorded in the carotid or femoral artery and a record of portal venous pressure was made by the manometer method described by McDowall (7) with a cannula in the central end of the splenic vein, the manometer-cannula system being filled with 5 p.c. sodium citrate. Pressure in the inferior vena cava was recorded, in some experiments, by the same method, with a cannula in the femoral or renal vein. In a few cases hirudin was given and was found not to influence the pressure changes in response to adrenaline or pituitrin.

Intravenous injection of 0·5 c.c. pituitrin invariably produced a fall

[1] The pituitrin used in this work was the extract of the posterior lobe of the pituitary prepared and issued by Messrs Parke, Davis and Co.

in portal pressure coincident with the rise in arterial, while the change in the inferior vena cava was variable and insignificant (Fig. 1).

Fig. 1. *PV*, portal pressure; *CA*, arterial pressure; *IVC*, pressure in inferior vena cava. Vertical lines mark time of injection of 0·5 c.c. pituitrin (Parke, Davis).

In many cases, more particularly after hirudin had been given, when the portal pressure had recovered from the temporary fall due to pituitrin, the new steady level was found to be slightly below that prevailing before injection of pituitrin, the difference being usually about 15 mm. citrate solution; this effect may conceivably be due to a return towards normal tone in the arterioles and capillaries of the mesenteric vessels as a result of pituitrin, for these vessels must invariably suffer some loss of tone as a result of the intra-abdominal manipulation necessary in these experiments. Fig. 2 shows that the same phenomena are seen in

Fig. 2. Upper record, portal pressure; lower record, arterial pressure.

response to vasopressin and that tachyphylaxis is evoked by repeated injections. In this experiment 0·5 c.c. pituitrin had been given 28 min. before the first injection of vasopressin. Oxytocin, however, is without

effect on either arterial or portal pressure, nor do repeated injections of this substance call forth an immunity to a subsequent dose of pituitrin. This is evident from Fig. 3 where 0·5 c.c. pituitrin was given 20 min. prior to the first injection of oxytocin and, despite four doses of the latter, a response to pituitrin was still obtainable; the magnitude of this response is such as might be expected about half an hour after the first pituitrin effect.

Fig. 3. Upper record, portal pressure; lower record, arterial pressure.

The fall in portal pressure following pituitrin may be due to: (*a*) constriction of arterioles or capillaries or both in the splanchnic area reducing the inflow to the portal vein or (*b*) diminished resistance to the blood flow through the liver, thus increasing the outflow from the portal vein.

Fig. 4. *C*, arterial pressure (zero at *BPO*); *P*, portal pressure (zero at *PPO*).

Figs. 4 and 5 at first sight appear to support the second of these possibilities, for it is seen that adrenaline and pituitrin are mutually antagonistic so far as the end effect on portal pressure is concerned.

Fig. 5. *PV*, portal pressure (zero at *OPP*); *FA*, arterial pressure (zero at *OAP*); *IVC*, pressure in inferior vena cava (zero at *OVP*).

A shows response to 0·5 c.c. adrenaline (1 in 10,000); B shows response to 0·5 c.c. pituitrin followed by 0·5 c.c. adrenaline.

Time of injection marked by vertical lines.

It seemed desirable, however, at this stage to determine the effect of pituitrin on the intrahepatic vessels alone, and for this purpose use was made of the cat's isolated liver, perfused by way of the portal vein with a mixture of defibrinated blood and 6 p.c. gum in Ringer at a constant pressure of 8 cm.; this pressure was chosen as being approximately that in the intact cat's portal vein. (The pressure recorded by means of a cannula in the splenic vein is usually about 6 cm. citrate solution, but by this method the whole of the inflow from the spleen is cut off, so that the recorded pressure must be below that in the intact animal.) The liver was enclosed in a suitably shaped plethysmograph so that volume changes could be followed, while the outflow from the inferior vena cava was recorded by means of an automatic tip. In all cases there was a steady increase in volume throughout the experiment, the rate of increase being greatest when the interval was longest between the death of the animal and the beginning of perfusion. Fig. 6 shows the effect (A) of injecting 0·2 c.c. pituitrin into the inflow tube at the portal vein; there is a temporary decrease in the rate of swelling and at the same time a slight diminution in the rate of perfusion flow. At (B) 0·2 c.c. adrenaline (1 in 10,000) was

injected, with the result that a considerable diminution of liver volume occurred; for the first few seconds the outflow increased but thereafter was greatly diminished.

Fig. 6. Upper record, liver volume; lower record, perfusion flow (each interval = 3·4 c.c.).

These records are typical of the results obtained in all experiments and indicate that pituitrin has a negligible constrictor action, while adrenaline has a very powerful one, on the intrahepatic vessels. It is also evident that this constrictor action of adrenaline, and not a swelling of the liver cells, is responsible for the reduced perfusion flow and appears sufficient to explain in large part, if not entirely, the rise of portal pressure produced in the intact animal. The injection of pituitrin and adrenaline together or of pituitrin during the action of adrenaline (Fig. 7)

Fig. 7. Upper record, liver volume; lower record, perfusion flow (each interval = 4·1 c.c.). A, 0·2 c.c. adrenaline (1 in 10,000); B and C, 0·2 c.c. pituitrin.

gives no indication of an antagonism such as appears in the portal pressure records given in Figs. 4 and 5.

The explanation of the apparent antagonism, therefore, is to be sought not in the liver but on the arterial side of the vessels feeding the portal vein. Adrenaline has a relatively greater effect on the intrahepatic vessels than on the small vessels in the splanchnic area, while the reverse is the case with pituitrin, probably because of the constriction of capillaries in addition to constriction of arterioles in the latter case (8). Indeed this difference in effect between adrenaline and pituitrin suggests that the main seat of action of the latter is in the capillaries, because in the liver where there are no capillaries in the true sense, but where the veins are sufficiently muscular to respond strongly to adrenaline, pituitrin produces but a feeble effect on the rate of blood flow. On the other hand, in the splanchnic area the action of pituitrin cuts off the supply of blood to an extent which lowers portal pressure even when the intrahepatic veins are constricted by adrenaline (Figs. 4 and 5).

Protocols are appended of liver perfusion experiments in which only the rate of perfusion flow was recorded.

Summary and conclusions.

1. The observations that adrenaline causes a rise and pituitrin a fall in portal pressure are confirmed; vasopressin is the principle responsible for the pituitrin effect.

2. The apparent mutual antagonism of these two substances is shown, by their effect on blood flow through the hepatic vessels, to be dependent on their relative effects on different parts of the splanchnic and portal systems.

3. Both adrenaline and pituitrin reduce the blood flow through the liver, the former mainly by its intrahepatic action and the latter by its constrictor action on the arteriole-capillary field. It is suggested that the effects of pituitrin may be chiefly due to the action on the capillaries.

I wish to express my thanks to Prof. Leathes for his continued interest in this work; to Dr J. W. Trevan for a supply of hirudin, and to Messrs Parke, Davis and Co. for samples of vasopressin and oxytocin.

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PROTOCOLS.

Liver perfused by portal vein; rate of flow recorded by automatic tip. Perfusion pressure 8 cm. water.

Exp. 1. Preliminary rate of flow 23·8 c.c. per min.
0·2 c.c. pituitrin; flow 22·1 c.c. per min. for 9 min.
0·2 c.c. adrenaline (1 in 10,000); flow increased to 30·6 c.c. per min. for 20 sec., thereafter 11·2, 8·8, 10·2, 13·6, 17·0, 20·4, 22·1, 22·1 in consecutive minutes.

Exp. 2. Preliminary flow 27·6 c.c. per min.
0·2 c.c. pituitrin + 0·2 c.c. adrenaline (1 in 10,000); flow fell to 20·4, 17·0, 20·4, 22·1, 23·8, 25·9 in consecutive minutes.
0·2 c.c. adrenaline (1 in 10,000); flow 20·4, 17·0, 20·4, 22·1 in consecutive minutes.

Exp. 3. Preliminary flow 13·6 c.c. per min.
0·2 c.c. pituitrin; flow 10·4, 10·4, 10·4, 10·4 in consecutive minutes.
0·2 c.c. adrenaline (1 in 10,000); flow 9·3, 6·8, 6·8, 6·8, 6·8, returning to 10·4 c.c. per min. 15 min. later.

Exp. 4. Preliminary rate 5·9 c.c. per min. (Swelling rapid.)
0·2 c.c. pituitrin; flow 5·9, 5·9, 5·9, 5·9, 5·9 in consecutive minutes.
0·2 c.c. adrenaline (1 in 10,000); flow 5·9, 4·2, 3·4, 3·4, 3·4 in consecutive minutes.

ADDENDUM.

Since this paper has been in the press the liver perfusion experiments have been repeated, using vasopressin and oxytocin instead of pituitrin. The results obtained with vasopressin were identical with those previously given by pituitrin, while oxytocin was without obvious influence on the liver volume or rate of perfusion flow.

ON THE LATENCY OF NEGATIVE AFTER-IMAGES FOLLOWING STIMULATION OF DIFFERENT AREAS OF THE RETINA.

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(*From the Department of Physiology, Oxford.*)

Introduction.

Although the after-images following sudden flashes of light have been the subject of careful investigations and measurements, notably by von Kries(16), McDougall(19), and Fröhlich(6), quantitative data regarding the after-images following longer lasting fixation of white or coloured objects are few in number and of a non-systematic nature. The earlier literature on the subject is mostly concerned with their rather complex phenomenology and its bearing on that particular theory of colour vision which in each case has inspired the investigation. It is not surprising, therefore, that cautious physiologists have been shy of attempting in this way to procure information about vision, and have turned to processes where quantitative work is at least as easy to accomplish and is more amenable to interpretation. (Cf. for example, Parsons(22), p. 114.)

Yet some experiments by Juhász(15) indicate that the latent period preceding the development of such after-images can be estimated with an accuracy sufficient for analysis. We therefore undertook a preliminary study of the problem, and, relating it to comparatively well-known anatomical and physiological facts, measured the latency of the negative after-image of a white object in different parts of the field of vision. One of us (R. G.) had recently been making comparable experiments in which "after-images of movement" were studied with reference to the area of retina on which the primary image fell(8, 9).

Our investigation is confined to the ordinary persistent after-image (McDougall's "secondary image"(19), some other writers' quaternary image), which is readily seen when, after fixation of any well-defined object, the gaze is transferred to a point on a uniform background. Purkinje(23) first described the rhythmical disappearances and re-appearances of these images, and their periodicity has been more recently

studied by Grünberg(10). Our earlier experiments were also directed to elucidate the factors influencing the periodicity, and it was only later that we were struck by certain features of the latency and determined to concentrate our attention on this latter problem. Our method of experiment was quite unsuitable for observing the much earlier momentary after-images following short flashes (McDougall's "recurrent images"), which, under the names of Hering's image, Purkinje's image (Bidwell's ghost), and the tertiary image, have been extensively studied by others. Some mention will however be made of them in connection with the interpretation of our results.

Method.

The experiments were carried out in a photographic dark-room in the laboratory, lighted by a single opal-glass electric lamp of about 80 candle-power at one end of the room. Before any observations were made, 20 to 30 minutes were allowed to elapse so that the observer's eyes might become adapted to the illumination. The condition of the eyes in this illumination may be described as one of moderate light-adaptation. Against the evenly black wall at the opposite end of the room from the lamp, white circular discs, provided with a fixation point, were observed binocularly. The discs were of paper coated with a mixture of zinc oxide and barium sulphate, and their illumination worked out at just over 5 metre-candles. The observer's eyes were 1·50 metres from the disc, and the time during which the disc was fixated was (in all experiments except where otherwise stated) 15 seconds. Before each fixation period the observer shut his eyes for 30 seconds in order to get rid of disturbing effects caused by after-images of the objects in the room.

The method of experiment was as follows. The experimenter was provided with a stop-watch, which he started while the observer sat on a stool with a chin rest in front of him. When the second hand reached 15 seconds the experimenter called out "Half a minute," and the observer closed his eyes. The experimenter gave a warning at 40 seconds, and at 45 seconds called out "On." The observer then opened his eyes and fixated the disc. At 60 seconds the experimenter lowered a screen (measuring 64 by 51 cm. and of the same white as the object) over a pulley, thereby covering the disc. The observer rapidly directed his gaze on to a fixation mark placed in the middle of the screen and so arranged as to be directly over the mark previously fixated on the disc. As soon as he saw the negative after-image appear, he said "On,"

and the experimenter noted the time. For the next observation, experimenter and observer changed places.

The lowering of the screen could be accomplished in half a second, a time short enough for most cases, as will be shown by the actual values of the latent periods measured. With practice on the part both of observer and experimenter, we believe that we have attained accuracy to within 0·2 second, but we are fully aware of the many technical improvements that could easily be introduced in these rather provisional arrangements. The early departure of one of us from England deterred us from embarking on a more ambitious programme.

Results.

Experiments with discs of constant size.

When two discs, each of 6 cm. diameter and with their centres 11 cm. apart, are used as object with the fixation point at the centre of one of them, an untrained observer will notice at once that the negative after-images of the two discs do not appear on the screen simultaneously. After a latent period of 2 to 3 seconds, that of the peripherally situated disc suddenly appears, but nothing is to be seen of the central after-image until a further interval of 2 or more seconds has elapsed. The first phase of the periodic after-image also ends in the peripheral image some seconds before the disappearance of the central image. To study this phenomenon in detail it is advisable to use smaller discs, and for most of our experiments we have employed discs of 1 cm. diameter (22′ visual angle). The after-images are more difficult to observe than those of larger discs, and some practice is required before accurate records can be made.

Fig. 1 illustrates an experiment of this kind with 1 cm. discs at varying horizontal distances from the fixation point. The visual angle subtended by the distance between the centre of the disc and the fixation point is plotted as abscissa, and the latent period of the bluish-black[1] after-image as ordinate. Three discs at different distances from the central fixation point were used simultaneously as stimuli and their after-images timed. The disadvantage of this method is that the exact time relations of two images rising almost together cannot be accurately recorded, but on the other hand more values can be obtained under similar conditions without disturbing effects of fatigue entering in. Experience has taught us that results are unreliable if either observer

[1] The light has a yellow tinge.

attempts to make more than about fifteen separate observations at a single sitting. Each value plotted represents the arithmetical mean of at least three observations, and care was taken that the discs were not

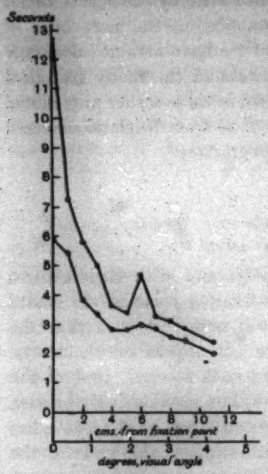

Fig. 1.

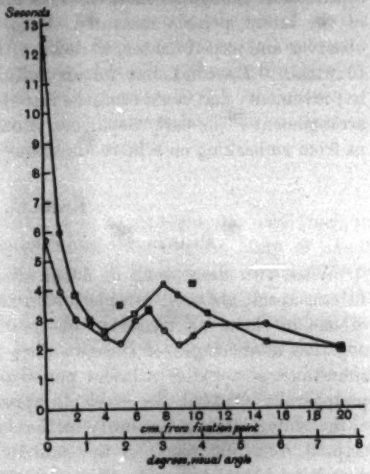

Fig. 2.

Fig. 1. Latency of negative after-images of 1 cm. (22′ visual angle) discs at varying distances from fixation point on horizontal meridian. Experiment of 21. v. 28; three discs used for each observation; each value plotted is the mean of three observations. Fixation period, 15 seconds. The upper curve is that of observer R. G.; the lower that of observer R. S. C.

Fig. 2. The same as Fig. 1. Experiment of 29. v. 28; two discs used for each observation. Fixation period, 30 seconds. Upper curve, observer R. G.; lower curve, observer R. S. C.

too close together. The smoothness of the curve drawn through the points bears witness to the reliability of the method. In the similar experiment shown in Fig. 2, only two discs at a time were used. (This series is one of the few in which we have fixated the object for 30 instead of the usual 15 seconds.) And in a third experiment of the same kind, a curve showing precisely the same features was obtained by observation of single discs.

Fig. 3 shows the same experiment for the vertical meridian. Each point represents the average of two measurements. The object in each observation was two 1 cm. discs, one with its centre either at 0 or at

1 cm. from the fixation point. The other was first moved progressively further out, and then back again towards zero. This set of observations was confirmed by another series taken a few days later. The dotted line

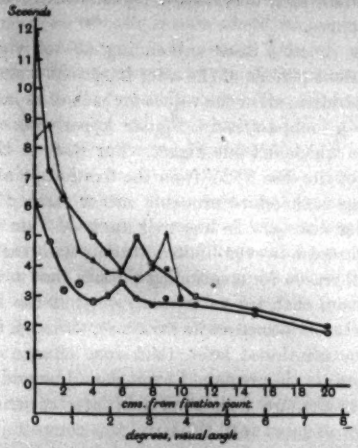

Fig. 3. Latency of negative after-images of 1 cm. (22′ visual angle) discs at varying distances from fixation point on vertical meridian. Experiment of 30. v. 28; two discs used for each observation; each value plotted is the mean of two observations. Fixation period, 15 seconds. Upper curve, observer R. G.; lower curve, observer R. S. C. Dotted line, untrained observer W. S. T. who was unable to maintain his fixation for discs more than 10 cm. from fixation point.

is the curve of a third untrained observer and is less regular than the others. He was in complete ignorance of the purpose of the experiment.

It will be seen that the latency of the after-image is longest in the fovea centralis, and that on passing out towards the periphery, it shortens on an almost perfect curve to a point at about 1° 50′ of visual angle. Then there is a rather sudden lengthening of the latency, which reaches a new maximum at about 2° 30′ from the fixation point. Passing further out towards the periphery, it again, though more gradually, becomes shorter. The second maximum, or "hump," was found with unfailing regularity in every experiment. Its position varied slightly from day to day, but was always between 2° and 3° from the fixation point. It was generally slightly more peripheral in observer R. G. than in observer R. S. C., and more peripheral still in a third subject W. S. T. Sometimes

it occupied a relatively broad region of the curve; sometimes the rise in the latency was restricted to a single point. An exploration of the "transitional area" between 1° 30′ and 3° with single discs in the horizontal meridian, each observation repeated three times, confirmed this part of the curve.

Using bigger (2 cm.) discs subtending 45′ of visual angle, and measuring the latent periods of the after-images at a series of points on the horizontal meridian, all of the values for each of us in one experiment fitted well into a comparatively regular hyperbolic curve, with the exception of one which fell out higher. For R. S. C. this reading was with the centre of the disc 3° 25′ from the fixation point, and for R. G. 4° 10′. The hump is therefore probably rather further out with larger than with smaller discs and is less well marked. Our observations on discs of this size are however limited to only two early experiments, and the principal reason for mentioning them is that it was the plotting out of one of them that led to the discovery of the hump. At that time we had no reason to suspect its existence, although it seemed likely, for reasons to be mentioned later, that some change of shape of the curve might appear in that region. Lest it should be said that suggestion influenced our results, we were careful in later experiments to avoid looking at our own figures until the series was completed.

Although struck by the regularity of our findings and its apparent relation to what is known about structural and functional differences between the corresponding areas of the retina, we naturally first considered the possibility of having timed different phases of the after-image on passing out from the centre to the periphery. About the latencies of the images of which the centres were within 1° of the fixation point, there could be no doubt whatever. Until the negative after-image appeared, there was nothing to be seen on the screen except sometimes a bright positive after-image which gave way to the gradually intensifying dark negative image. The after-image of a 1 cm. disc with the fixation point at its centre differed from the others in being much fainter (as also was its primary image), and this sometimes led to its being timed too late or occasionally to its failing to appear at all. With discs more than 1° from the fixation point, R. G. sometimes noticed a bluish image on the screen just as it fell down, *i.e.* with a latency of less than 0·5 second. This image in most cases rapidly vanished, giving place to a short indefinite positive image followed by the ordinary black negative after-image (with a minimum latency of 1·8 seconds). On rare occasions this rapidly vanishing after-effect was also seen by R. S. C.

and W. S. T. There is some doubt as to its nature which will be discussed again in the account of the next experiments. But in the present connection, its nature is less important than the question whether it has interfered with our timing of the first negative phase. Apart from the fact that only one observer was certain about its appearance, it seems to be too early and momentary to be able to interfere with any images other than the most peripheral ones. Besides, it was so faint and rapid with 1 cm. discs as to be scarcely noticeable, whereas the ordinary negative after-image outside the fovea was so black that it could not get lost during the 4 seconds or more of its first phase. It is also significant that the latencies continue to get shorter for some distance outside the point at which the momentary image first appears; the position of the hump, in fact, cannot be exactly correlated with its appearance.

An adequate explanation of our findings can scarcely be attempted at present, but the lines along which it is to be sought for are certainly indicated by the structural differences between the retinal areas tested. The transitional area in our curves appears at a visual angle where, according to Hering(13) and others, the retina changes its structure from that of an organ dominated by cones to a rod organ. The wholly rod-free area is smaller—perhaps restricted to the fovea, *i.e.* about 1° of visual angle (Parsons(22), p. 13), or somewhat bigger (about 2° horizontally) according to functional tests by von Kries and Nagel(17) and by Fröhlich and Vogelsang(7). The careful experiments of Abney and Watson(1) make it highly probable on functional grounds that individual variations in the extent of the rod-free area are to be found, and apparently there is even a small class of persons with rods scattered throughout the macula. But outside an area subtending about 4° of visual angle (corresponding to 2° of the abscissa in our curves, since we are only taking account of the radius), the rods suddenly come into play in greater force than the cones. If this be so, the sudden rise in our curves probably indicates that, as it were, a new organ is now reacting to the stimulus, and that either the latency of the after-image of an object whose primary image falls on rods in this area is longer than that of one falling on cones, or there is some more complicated interaction between the two structures leading to a lengthening of the latent period. Evidence that interaction does occur will be adduced in the next section.

That the hump varies slightly in its position, in its steepness, and in its extent, from day to day seems to be well accounted for by the varying condition of the observers' eyes. Although time was always

allowed for adaptation to the illumination of the room at the beginning of each experiment, the conditions were probably not really identical on dull days and on days of brilliant sunshine. Burch(3), esp. p. 212) has shown that the after-effects of bright light last much longer than is generally supposed. The intensity of the primary stimulus may also have varied slightly from day to day, as the ordinary town supply of electricity was used to feed the lamp. That the hump should be less sharp with larger than with smaller discs is also consistent with the explanation outlined above, for the transition from cone organ to rod organ (or mixed organ) will be less abrupt in the former than in the latter case.

Even if the rod-free area is confined to the fovea, at least the first three points plotted in Figs. 1, 2 and 3, *i.e.* those of 1 cm. discs with centres at 0, 1 cm., and 2 cm. from the fixation point, are values for after-images corresponding to primary images falling wholly on cones. The initial steep fall is therefore exclusively a cone curve, and is indicative of functional differences between the cones in the centre of the fovea and those further out. Other evidence pointing in the same direction has been brought forward by French(5) and by Heinz and Lippay(11), who state that the threshold of difference of brightness is higher in the centre than at the edge of the fovea. It is tempting to imagine that to the right of the hump the cone curve is replaced by a rod curve of similar hyperbolic shape.

Finally a few remarks must be made about the phenomenology of these images. The appearance of momentary negative images of extremely short latency, and of positive images preceding the first phase of the ordinary negative image, has already been described. Both the primary image and the after-image of a disc in the centre of the field of vision differ from all others in that they are fainter, and the after-image has fewer phases. This is no doubt due to the inferior sensitivity to light of the fovea as compared with the rest of the retina. On the other hand the contour of a peripheral after-image is naturally less distinct than that of a central one. Black negative images always tend to be surrounded by a bright halo. Phases after the first are progressively fainter and have less distinct contours. The development of the first negative phase shows interesting alterations—perhaps best observed with a 2 cm. (45′) disc—on passing out from the centre of the field of vision. Images close to the fixation point rise very gradually to a maximum of blackness; those in the "transitional area" begin in a similar gradual way, but then a sudden intensification occurs and the

plateau is reached at once; further out still no slow rise is found, but the image suddenly appears in its maximum intensity[1]. In every case the disappearance of the first phase is equally sudden. It is always interesting to compare the sensations evoked in consciousness by appropriate stimuli with the mechanical responses of the muscles of an unconscious animal in purely reflex action. The slow rise of the central after-images (? cone response) may be regarded as analogous to what Liddell and Sherrington(18) have called "recruitment" in reflex action; while the sudden rise (? rod response) is reminiscent of the "*d'emblée*" type of reflex.

With 1 cm. discs, the primary image at points more than 2° from the fixation point frequently pops in and out during the 15 seconds' observation period. This fact was studied by Aubert(12) and by Holth(14), and is probably essentially the same as that described by Dunlap(4). In one observation at 7° 30′ from the centre of the field of vision, one of us estimated that the primary image disappeared in this way for more than half of the 15 seconds' fixation period. The after-image was, however, as intense and distinct as ever, and appeared with the usual latency. It therefore seems that the after-image is not dependent on the perception in consciousness of the primary object, but is conditioned merely by previous excitation of some part of the retino-neural apparatus.

Experiments with discs of varying size in the centre of the field of vision.

The observations detailed above suggest many further simple problems, but the time at our disposal has not allowed us to tackle systematically more than one. This was to investigate the relation between latent period of after-image and size of disc, other conditions being kept constant. Juhász(15) gives a few figures showing that the complementary after-image of a red object appears after a shorter latency with large objects than with small. Our findings confirm and amplify his.

In making these observations, we were going back to a type of experiment which, at the beginning of our work, we repeated many times for the sake of practice, but which we did not, at that time, think simple enough to continue. When reconsidering the first complete series of these preliminary observations, in which the fixation point was always

[1] Since the above was written, a communication by Walther(25) has come to our notice. He too records the same difference between the development of a central after-image and that of a peripheral one, and mentions the fact that the former are less intense in colour.

at the centre of the disc, it was found that by plotting the visual angle subtended by the *radius* of a disc as abscissa and the latency of its after-image as ordinate, a curve similar to the one for small discs at different degrees of eccentricity was clearly indicated. The first falling part of the curve flattened out at about the same visual angle and was followed by a rise, but as we had not used discs big enough to cover the whole range corresponding to the curve for small discs, we were not in possession of the requisite data for deciding whether the resemblance was complete, *i.e.* whether a renewed shortening of the latent period occurred beyond 3° from the fixation point. We therefore decided to repeat the experiments with discs up to 22 cm. in diameter. Here again, as shown in Fig. 4, which gives the arithmetical means of three series, we met with higher values for the latencies in a "transitional area" between 2° and 3° of visual angle subtended by the radius of the disc. Beyond this point the latencies became shorter. Six separate experiments all supported these findings, and the height of the hump was frequently (as in Fig. 5) more than 1 second. By taking averages of a number of experiments a smoother curve is obtained, but the hump tends to be flattened out since its size, position, and extent vary slightly from time to time just as they do with the small objects. It is immaterial whether the observations are made with series of increasing size, or of decreasing size, or whether the discs are taken in haphazard order. Two experiments in which the visual angle was altered by varying the distance of the observer's eyes from discs of constant size, were less satisfactory but still showed evidence of the hump.

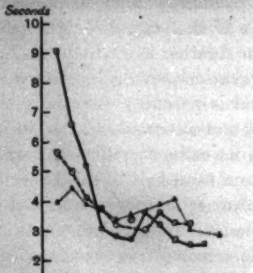

Fig. 4. Latency of negative after-images of discs of varying diameter with the fixation point at their centres. Continuous line, observer R. G.; broken line, observer R. S. C. Each value plotted is the mean of three to five observations from the experiments of 8. vi. 28, 11. vi. 28 and 12. vi. 28. Fixation period, 15 seconds. Dotted line, untrained observer W. S. T. Means of two to four observations from experiments of 6. vi. 28 and 13. vi. 28.

Suggestion can, we believe, be eliminated as a factor in our records by three considerations. (*a*) When attention is concentrated on seeing the first sign of the after-image, it is impossible to judge time accurately, and the observer was afterwards often surprised to find how mistaken

he had been in his estimate of the relative lengths of some of his periods. (*b*) We were careful not to look at our own records until each experiment was completed. (*c*) Fig. 4 shows two experiments in which a third untrained observer, who was quite ignorant of the results we were expecting, took part. His curve bears a certain resemblance to his other curve with the small discs (Fig. 3), and is unlike both of our own. The peak of his hump occurred with the 18 cm. disc in each of the three experiments of the present type at which he made observations.

A number of interesting deductions can be drawn from these findings. If we take the curve drawn as a continuous line in Fig. 4 as paradigm, the behaviour of the after-image of the 12 cm. disc (2° 17′ radius) is worthy of attention. The whole image invariably appears at the same moment, although the steep descent of the curve from 2 cm. to 8 cm. might lead one to expect that the latency of its central part would be considerably longer than that of its edge. The process of development of the after-image does not seem to need more than a start from the periphery in order at once to be set going all over the area previously stimulated. Since the central area can be regarded functionally as a blind spot in this experiment, the phenomenon is exactly comparable with that filling up of the blind spot corresponding to the optic disc, which, in normal vision, has led to so much investigation and discussion. The facts may be expressed as a general law by saying that **the latency of the after-image of the disc as a whole is that corresponding to the region of retina on which the image of its edge falls.**

A further important application of this rule is seen in the case of discs of 14 cm. and 16 cm. diameter. Their edges fall in what we have termed the transitional area, where the latent period is longer than that in areas more central and more peripheral. One might anticipate therefore that the after-image would first appear as a ring of about 12 cm. diameter and then, either gradually, or perhaps at once, spread inwards and outwards until the disc was complete. In practice nothing of the kind occurs. There is no sign of a negative after-image at the end of the latent period characteristic of a 12 cm. disc. Not until 1 second or so later does the after-image appear, and it is seen from the first as a complete dark disc. The edge seems to be the determining factor, and the after-effects in consciousness of previous stimulation of areas of retina within that edge seem to be inhibited for about 1 second. Whatever the explanation may be—whether psychological or physiological, if such a distinction be of any value—the finding as such illustrates the great importance of contours in vision, and also sheds light on the means

employed to overcome the difficulty of possessing in the retina two structures (rods and cones) of different sensitivity and functions, and to combine them in use as a single sensory organ. For if we were right in our previous argument in interpreting the hump as due to the excitation of rods, we have here in the transitional area the rod mechanism coming into action in overwhelming force and actively inhibiting the after-effects which would otherwise have followed stimulation of cones. Such an assumption is not altogether new. It has been adopted by P. von Liebermann and by Müller(20,21) to account for some experiments on the foveal scotoma in scotopic vision, and one of us(8,9) has used it to explain some observations on the after-effect of seen movement.

It has been seen that, so far as their latent periods are concerned, the behaviour of the after-images of discs of varying size bears a close resemblance to that of after-images of small discs at varying angular distances from the fixation point, and that the visual angle subtended by the radius of the former can be correlated with the degree of eccentricity of the latter. The same is true also of their phenomenology, which is very much easier to observe with these large discs than with small. Positive images were frequently (but not invariably) seen both before the first negative phase, and between later negative phases, with all sizes of disc. Large discs gave after-images with more phases and a longer total duration than did small (cf. Juhász(15)), but their contours were less sharp. Phases after the first were always less black and less well defined. Frequently they were also patchy, but it is noteworthy that the dark patch invariably included a considerable length of edge and was never in the middle of the field previously occupied by the disc. In the third and later periods it was rather unusual for the image of the whole disc to be visible throughout, but some part of the edge was always the first portion to appear and the last to disappear.

Observer R. G. saw the early momentary image or ghost (described in the last section) with great regularity in these experiments. It was not present with small discs, but appeared as soon as the radius reached a visual angle of 1° 30′, and then became darker and clearer with further increase in size of the disc. It was therefore seen central to the transitional area, and persisted throughout this region and beyond it. With the larger discs employed, the image actually timed was separated from the ghost by a much shorter interval than when observations were made in the transitional area. Sometimes with very large discs the two almost appeared to overlap. It is clear then that the hump on our curves cannot be dependent on timing the ghost in some cases and the ordinary

negative image in others. Even if, for purposes of argument, we count the ghost as R. G.'s first period, the second period now shows the hump, which, as we believe, ought rightly to be ascribed to the first. Other observers occasionally saw the ghost with big discs. As to its nature, there is every reason to believe it to be a characteristic of rod vision—perhaps Purkinje's image, which, as Fröhlich and Vogelsang(7) have lately shown, always jumps the fovea. So soon as the primary image falls on a sufficient number of rods, it is seen; but this number is less than that required for the renewed lengthening of the latent period in the transitional area.

Discs of less than 8 cm. diameter show the slow development of maximum intensity in the first phase of the negative after-image that was described as characteristic of areas dominated by cones; while those of more than about 16 cm. diameter give a *réaction d'emblée*. In the transitional area a stepped rise was noticed—at first a gradual process of development, terminated by a sudden jump to the full intensity. The preliminary slow rise was cut short by the second intensification earlier with larger than with smaller discs. This is a matter which requires a few words since it leads to complications in timing the first appearance of the after-image. The surprisingly long latencies which one of us recorded on some days for the smaller discs, we are inclined to ascribe to a relative insensitivity to the feeble early part of the stepped rise. We have made attempts in a few experiments to record at the same time both the beginning of the rise and the point when full intensity is reached. Fig. 5 shows one of these. The intervals given are probably too long for the discs of nearly 14 cm. diameter, where the stepped rise was replaced by a simple abrupt beginning. It is difficult to call out and to record the second point when it follows close on the first, so that the upper of the two curves probably ought to be rather lower. But the hump is quite definite in both. In some of the other experiments R. G. attempted always to time the very first sign of the after-image, and R. S. C. the point at which maximum intensity was reached. The general features of the two curves were always the same.

Experiments with discs of varying size in the periphery of the field of vision.

When experiments of the kind described in the last section were made with the centre of each disc 11 cm. (4° 12′) to one side of the fixation point, the results, as shown in Fig. 5, were entirely different. The latent periods for discs of all sizes were the same, within 0·4 second

for one observer, and 0·2 second for the other. In an earlier series, although this remarkable constancy was not attained, there could be no doubt that the interpretation was the same.

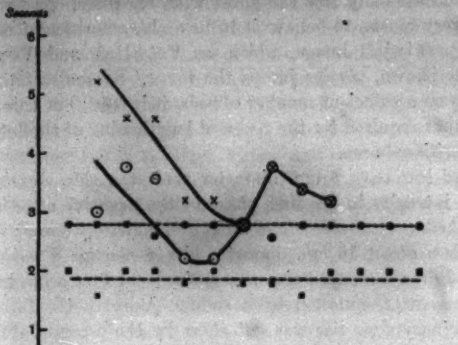

Fig. 5. Continuous line, discs of increasing size in centre of field of vision. Experiment of 8. vi. 28; observer R. S. C. Circles, latencies of first appearance of after-image; crosses, attainment of maximum blackness. Single values. Full description in text.
Broken lines, discs of decreasing size with centres 11 cm. (4° 12′) from fixation point. Single values from experiment of 13. vi. 28. Circles, observer R. S. C.; squares, observer R. G.

The conclusion that the after-images were all essentially alike was further supported by their appearances. R. G. saw a "ghost" before all of them, but it was more distinct and lasted longer with the larger objects. On one of the days R. S. C. saw it consistently too, and it made the timing difficult because it seemed to run into the first phase of the ordinary after-image. The development of all the images was equally sudden. The total duration of big ones was longer than that of small, and their contour was better defined. The 2 cm. ones had such indefinite boundaries that they were difficult to observe accurately and appeared merely as dark smudges. One of us noted that the after-images of small discs were blacker than those of large. This may have been an effect of contrast.

In these experiments the images of discs of 20 cm. and more in

diameter must have fallen partly on the fovea, and when the diameter was more than 22 cm. they overlapped the fixation point. The facts that the latency was not then lengthened and that the after-image was seen as a complete whole from the moment of its first appearance, afford further evidence (*a*) of the filling in of a contour across a functional blind spot, and (*b*) of the rods being the controlling power when a large number of rods and a small number of cones are stimulated together. If the conditions were reversed, it might perhaps be possible to obtain evidence of an extensive stimulation of cones inhibiting the after-effects of a restricted stimulation of rods, but we have no evidence on this point.

Discussion.

Two important principles in the physiology and psychology of vision are well illustrated in the experiments described above. One is the importance of form, exemplified (*a*) by the filling in of areas and contours in a blind region of the field of vision, and (*b*) by the observation that the length of the latent period preceding the development of a negative after-image is dependent on the region of retina on which the edge of the primary image falls, and is independent of the position of other parts of the image. An experiment of a slightly different kind is also of interest in this connection. It was mentioned earlier that when the centre of a 6 cm. disc is used as fixation point and a second similar disc is put with its centre 11 cm. to one side, the after-image of the latter appears at least 2 seconds before that of the former, and the periodicities of the two images are independent. If the two discs are now combined into a single figure by filling the intervening space with a white strip 6 cm. broad and the observation is repeated, the appearance of the negative after-image varies with different observers. R. G. sees at first only the dark image of the peripheral end of the object; the blackness then sweeps across like a shutter until the whole figure is complete; but at the conclusion of the first phase both ends disappear together. R. S. C. sees the outline of the whole figure from the moment the image becomes visible, but the periphery is at first darker than the end fixated; both ends fade simultaneously. Two other observers reported that at the very beginning of the first phase they saw the complete figure of uniform blackness and with sharp outline, but that it faded from the distal end inwards. As is only natural, the existence of a contour uniting the two discs therefore modified in each case the appearance and time relations of the after-images evoked by central and peripheral stimulation, and to a greater or less extent synchronised them. An interesting

series of rather similar observations has been reported by Rothschild (24).

The second principle is the interaction which occurs between the various receptors stimulated by a primary image. In our experiments this manifested itself as inhibition of the cone mechanism by the rod mechanism. We have no information as to the anatomical site of the process, but in view of the recently published work of Adrian and Matthews (2) on interaction of neurones in the conger eel's eye, it may well take place in the retina.

On the factors other than the area of retina stimulated, which influence the length of the latent period, our evidence is for the most part of a negative kind. Juhász (15) found that the latency shortens as the size of the disc increases. Our experiments show that this is approximately true when the centre of the disc is the fixation point, but that when the centre of the image is outside the macula, the areal extent of the primary image is devoid of influence. In other words, the *number* of receptors stimulated is of no significance (at any rate with discs subtending more than 22′) provided the edge of the image falls predominantly on receptors outside our "transitional area." The changes in latency with discs in the centre of the field of vision are due, not to any effect of spatial summation, but to the fact that the edges are falling successively on receptors of changing character.

Juhász also says that within wide limits the latent period is not dependent on the intensity of the primary image, and that for short durations of the primary stimulus the latent period shortens as that duration is lengthened. With both of these statements our findings are in complete agreement. A comparison of Figs. 1 and 2 shows that for the cone part of the curve the latencies after 30 seconds' fixation are consistently shorter than those after 15 seconds' fixation. On two occasions we have measured the latent periods with various discs under the usual and under greatly increased illumination. On one occasion the illumination of the object was increased tenfold by using a second lamp. The experimenter interposed his shadow between this lamp and the screen at the end of the fixation period, so that the field on which the after-image was projected remained constant. The latencies of the after-images of five discs, the smallest 2 cm. and the largest 18 cm. in diameter, were not sensibly affected, though, as between the various discs, they ranged from 2 seconds to 9 seconds. The actual figures are given below, those in brackets being for the strong illumination. Their standard of accuracy is probably not as high as usual owing

to the disturbing conditions of experiment for observer and experimenter.

	2	4	8	14	18 cm. diam.
R. G.	8·0 (6·0)	4·6 (4·6)	2·0 (1·8)	2·8 (2·8)	2·8 (3·0) seconds
R. S. C.	5·4 (6·2)	(2·4)	2·2 (2·2)	2·4 (3·0)	2·6 (2·4) seconds

On the other occasion, the illumination of both object and screen was increased threefold by bringing the lamp nearer. Unfortunately only two discs were observed, but the result was as follows:

	2	4 cm. diam.
R. G.	9·0 (7·6)	7·0 (7·0) seconds
R. S. C.	6·0 (6·0)	4·0 (4·2) seconds

These rather fragmentary observations (strongly supported however by Juhász) are mentioned because they make it highly improbable that variations in the size of the pupil influence the latency of after-images. In any case such variations in the pupil in response to light cannot, of course, explain the hump in any of our curves, nor indeed any part of the curve in Figs. 1, 2, and 3.

Summary.

1. The latent periods preceding the development of the negative after-images of white circular discs have been measured under known experimental conditions. Using small discs of constant size at varying distances from the fixation point, the latent period is longest in the centre of the field of vision, shortens rapidly to a point about 2° from the fixation point, then increases to a new maximum between 2° and 3° from the fixation point, and thereafter again shortens.

2. The second maximum or "hump" in the curve is interpreted as being due to the rods replacing the cones as the dominant receptor organs in and beyond this area. Changes in the appearance of the after-image lend support to this idea.

3. The after-images of discs of varying size in the centre of the field of vision were next investigated, and it was found that a precisely similar curve was obtained by plotting the visual angle subtended by the radius of the disc as abscissa and the latent period as ordinate. The latency of the after-image of the disc as a whole is that corresponding to the region of retina on which the image of its edge falls.

4. This finding is a striking illustration of the importance of contours in vision, and also affords evidence of inhibition of the cone mechanism by the rod mechanism. Observations on the phenomenology of the images amplify and confirm the argument.

5. The latent periods of the after-images of discs with their centres in the periphery of the field of vision are (within wide limits) independent of their size.

6. The factors which influence the length of the latent period are briefly discussed.

Mr W. S. Tegner, Scholar of New College, kindly acted as observer in a number of these experiments.

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AN APPARATUS FOR THE CONTINUOUS RECORDING OF THE OXYGEN CONSUMPTION OF SMALL ANIMALS.

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This apparatus was designed for use in a projected study of the O_2-consumption of small animals after dosage with thyroxine and related compounds. Because of the persistence of the action of a single dose of thyroxine, it was highly desirable that several experiments should proceed at the same time. Hence the apparatus should be compact, easy to operate, and inexpensive. The arrangement which we describe meets these requirements; it also permits graphic registration of the O_2-consumption and of the movements of the animal. The animal is placed in a closed chamber and the carbon dioxide and water which it produces are absorbed by soda lime. Oxygen bubbles in through paraffin oil to keep the pressure constant in the chamber, and the entrance of each bubble is recorded on a drum. The oxygen consumption is determined from the rate of formation of the bubbles.

Description of apparatus.

One form of the apparatus for a guinea-pig or very small rabbit is shown in Fig. 1. Fig. 2 is a detailed drawing made to scale.

The chamber. The chamber is a rectangular museum jar $8\frac{1}{2}$ in. long, 4 in. wide and $8\frac{1}{2}$ in. high. It rests on a brass plate $\frac{1}{4}$ in. thick into which are screwed 6 upright brass rods ($10 \times \frac{5}{16}$ in.) threaded at the upper ends to receive the wing nuts by which the cover is fixed to the chamber. The cover is a brass plate like the base, its lower surface faced true in the lathe, pierced by six holes corresponding in size and position to the uprights from the base. A rubber gasket between cover and jar makes the joint air-tight: a mat of spongy "sorbo" rubber under the jar equalises the strain. The whole chamber is placed in a thermostat below the surface of water.

The absorption apparatus. The ventilating apparatus is attached to the cover. It consists of a fan[1] rotated in the upper part of a cylindrical

[1] Small fan blades were obtained from A. C. Gilbert and Co., 57 Kingsway, W.C.2.

soda-lime container so that the chamber air circulates through this continuously. The fan shaft bears a collar which rotates in a mercury-filled

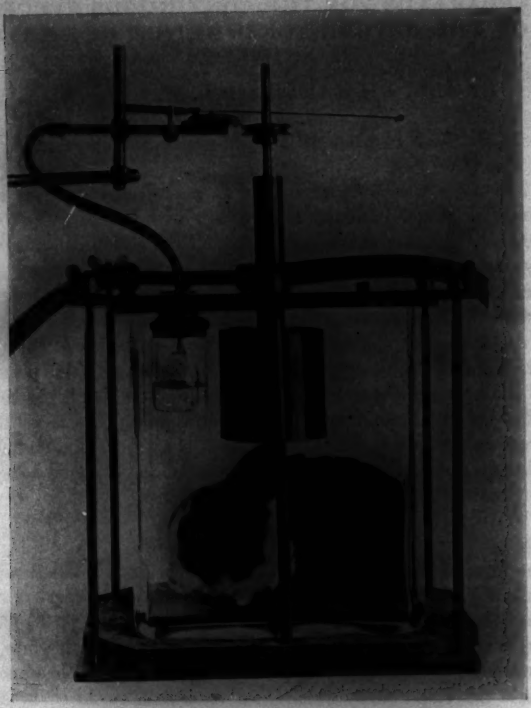

Fig. 1. Assembled apparatus with rabbit weighing 375 gm.

cup within the neck of the soda-lime cylinder, thus making an air-tight mercury seal. The mercury is covered with a layer of liquid paraffin. This lubricates the mercury and prevents the leakage of air between the mercury and the brass. Such a seal has been in use for a year without giving trouble of any kind.

The oxygen recorder. The inflow of oxygen necessary to maintain a constant pressure within the chamber is recorded by means of a valve.

This must be very rigidly made. The valve that has been used consists of a glass bottle cemented to a brass collar, which is threaded on the inside

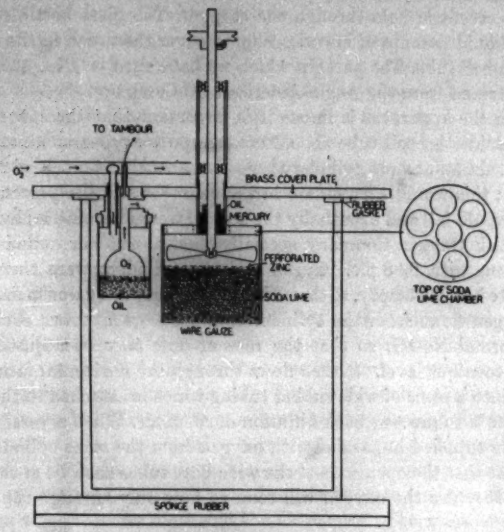

Fig. 2.

for attachment to its stopper. The latter is made of solid brass and is threaded at the bottom to receive the bottle, and at the top so that it may be screwed either into the cover of the chamber or into a solid brass bracket fixed below the water surface to the side of the thermostat bath. Two $\frac{1}{4}$ in. holes are drilled vertically through the stopper, one in the centre, and the other midway between the centre and the circumference. A horizontal hole is drilled from the circumference into the central hole at a point midway between the two threaded ends. A short piece of thick-walled brass tubing is soldered into the upper end of the central hole for the connection of this with a tambour. When the valve is fixed to the thermostat bath instead of to the cover of the chamber, a piece of wider brass tubing is soldered into the horizontal hole and this is connected with a hole in the cover of the chamber by means of a piece of rubber tubing about 4 ft. 6 in. long and having an internal diameter

of $\frac{1}{8}$ in. Into the lower end of the central hole is cemented the stem of a glass bell tube, the edge of which is notched at one point with a round file and polished. The perpendicular arm of a brass T tube is fixed air-tight into the eccentric hole through the stopper. The glass bottle contains enough liquid paraffin of low viscosity to cover the notch in the edge of the glass bell tube. The paraffin which we have used is "No. 4 Cosmetic Oil," obtained from the Anglo-American Oil Company.

When the apparatus is in use it is important that the space in the valve outside the bell tube should contain pure oxygen at atmospheric pressure. In an oxygen cylinder there is frequently as much as 2 p.c. of nitrogen; this would accumulate in the chamber and the percentage of oxygen would fall and eventually the animal would become asphyxiated. The British Oxygen Company specially washed out our cylinders and filled them with 99·8 p.c. oxygen and the cylinders were then never allowed to become empty, so that a high degree of purity was maintained. The oxygen flows from the cylinder through a wash bottle containing twice normal NaOH, so that the rate of flow may be adjusted to a roughly constant level. It then flows through the horizontal arm of the brass T into a piece of wide rubber tubing which is attached to the other end of the T to prevent back diffusion of room air. When several such T pieces are supplied in parallel with oxygen from the same cylinder, it is important that the open ends of the wide-bore tubes shall be at the same level. Otherwise the oxygen will tend to flow only through the lowest opening.

The interior of the chamber is in communication with the space within the glass bell tube: as oxygen is used by the animal, the fluid in the glass bell tube rises until the notch is uncovered and a bubble of O_2 admitted. The notch makes each bubble enter at the same place, under the same glass surface and ensures uniformity in the size of the bubbles. This result is not achieved unless the valve is always adjusted to the same level, and if the valve is attached to the cover of the chamber as in Fig. 1, the whole apparatus must on each occasion be levelled, and kept level. For this reason it has recently been found more convenient to have the valves permanently attached to the side of the thermostat bath and to connect them with the chamber by means of rubber tubing, as above mentioned. The entrance of each bubble is recorded on a slowly moving drum by the tambour which is connected with the tube projecting from the brass stopper.

Thermostat. An advantage of the small size and compactness of the apparatus is that several chambers may be placed in the same thermostat

and their fans driven by the same motor. For this purpose a wooden box lined with zinc and large enough to hold four chambers has been used. The water is kept well stirred by a fan, and its temperature is maintained at 25° C. by a large toluene regulator, the mercury column of which makes an electric circuit working a relay, which cuts off the current supplying the heating unit. The latter is a long carbon filament bulb as used in an electric stove, covered with foil to prevent direct radiation from reaching the chambers. A condenser of capacity 1 microfarad is connected across the high voltage terminals of the relay to prevent the formation of arcs. It is important that the temperature should be extremely constant since a variation of 0·01° would produce a definite distortion of the record.

Tests and calibration.

When the empty chamber is placed in the thermostat, and the fan is started, the air expands rapidly at first and bubbles pass outwards through the valve. After about 30 min. the tambour should record a horizontal straight line whether the pressure inside is equal to the atmospheric pressure, or is made to differ from it by such a slight amount as will not cause bubbles to pass in either direction through the valve. The apparatus forms a very sensitive barometer, and small changes in the air pressure in the room, due to such causes as the opening and shutting of doors, are apt to disturb the record unless there is free communication between the air in the room and the air outside. The most usual source of leaks is the tambour, and this should be tested daily.

In calibrating the apparatus the chamber is connected, through a special hole in the cover, with an aspirator filled with mercury and 50 c.c. or 100 c.c. of this are allowed to flow into a volumetric flask, while record is made of the oxygen bubbles which enter to replace the air thus withdrawn. The temperature of the aspirator is taken at the time of making the test, and an appropriate correction applied to the volume of air withdrawn. It has been found that if the total volume of air contained in the chamber and the aspirator is very large, the size of the bubbles is slightly increased. Hence the volume of the air in the aspirator should be small. The time taken for the formation of each bubble may vary between 10 and 30 sec., and it has been found that increasing the rate of bubbling slightly increases the size of the bubbles so that, for accurate work, a calibration curve should be used for each chamber and its corresponding oxygen valve, relating the volume of the bubble to varying speeds of bubbling. For most purposes,

however, it is sufficient to calibrate the apparatus at a mean rate of bubbling, and to assume that the volume of the bubbles is constant. The error so introduced is usually less than 2 p.c.

Calibrations have been performed both on days when the barometer has been high, and on days when it has been low and the volume of the bubble appears to be independent of the barometric pressure. However, when the barometric pressure is rising, air passes into the chamber even when no air is being withdrawn by the aspirator; when the barometer is falling, the reverse process occurs. A suitable correction may be applied as follows: Accurate barometric readings are taken at frequent intervals, and the change occurring during the calibration is calculated from the smooth curve drawn through the observed points. If V is the volume of the chamber, P the barometric pressure and δP the increase of P occurring during the experiment, then a quantity $V\,\delta P/P$ is added to the volume of the mercury withdrawn. The amount of this correction may be reduced to a negligible value by reducing the size of the chamber. Four of these chambers have been in daily use for some months. Calibrations performed at intervals have usually given quite concordant results, though occasional unexplained variations have occurred. The extreme degrees of such variation from the mean in the four cases were 3·3 p.c., 2·3 p.c., 2·5 p.c. and 5 p.c. The accuracy indicated by these results is not high, but the uncontrolled variations which occur in the animals themselves are such that greater accuracy of measurement would be of little value.

The measurement of oxygen consumption.

When an animal is placed in the chamber, its respiration is recorded by the tambour. This is presumably due to the rise and fall of the pressure of the air in the chest. Other movements are shown by sudden large distortions of the record, and also by irregularities in the rate of bubbling. These may be due to exaggerated respiratory movements with the glottis partially closed; they may also be due to sudden fluctuations of temperature (see Fig. 3). The method thus has the great advantage that it is possible to tell without difficulty which parts of the record correspond to periods during which the animal was at rest, and to obtain an estimate either of the basal, or of the total, metabolism.

When an animal is accustomed to the apparatus, it usually makes only slight and infrequent movements during the greater part of the experiment. Occasionally it becomes more restless and its movements are recorded together with an increase in its consumption of oxygen.

If the movement has lasted for some time, the increase in the oxygen consumption may persist for some time after the movement has ceased.

Fig. 3. From the top downward:
1. Record of calibration.
2. O_2 consumption record of a small rabbit while quiet, with exception of twice lifting head.
3. Rabbit restless, cleaning himself and moving legs.
4. Time in minutes.

The following figures are taken from an experiment lasting 2½ hours on a rabbit weighing 550 grm. The periods chosen are those in which the record showed only slight and infrequent movements of the animal.

One bubble of $O_2 = 1{\cdot}43$ c.c.

Time	Min.	Number of O_2 bubbles		O_2 bubbles per min.
3.30–3.40	10	51		5·1
3.41–3.47	6	29		4·83
3.52–3.58	6	28·5		4·75
4.05–4.14	9	44·5		4·9
4.42–4.47	5	26		5·2
4.54–5.09	15	73		4·87
5.11–5.17	6	30		5·0
5.19–5.29	10	48		4·8
	67	330	Average	4·92

This corresponds to 7·03 c.c. per min. The barometric pressure should be recorded during the experiment, and if it is changing rapidly, a correction should be applied as in the case of calibration. The volume thus obtained should be reduced to a standard pressure.

For larger or smaller animals other museum jars may be chosen, and in such case the dimensions of the base and cover would be correspondingly altered. In many ways a solid metal chamber would be more

satisfactory. In any case maximum accuracy will be attained if the jar is no larger than that which just accommodates the animal in comfort. The volume of oxygen admitted in each bubble can easily be altered since it is dependent on the cross-section of the bell tube, and on that of the bottle into which it projects, and on the quantity of fluid. The apparatus has now been used for about a year in a study by J. H. Gaddum of the effect of thyroxine and allied compounds on the oxygen consumption of rats. The results of this work, an account of which will be published in due course, have been such as to encourage the belief that the apparatus will be of service in the study of other similar problems.

Acknowledgment is due to Dr Dale for revising the original description of the apparatus, written more than a year ago, and to Dr Gaddum for the privilege of including results of his experience with it in the meantime.

THE EFFECT OF HYPER-RESPIRATION ON THE BLOOD-PRESSURE IN MAN.

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Introduction.

As a result of a preliminary series of experiments using a simple combination whereby it is possible to record continuously variations in human blood-pressure (Thompson(5)), we thought it would be interesting to confirm the work of Vincent and Cameron relating to the influence of hyperpnœa on the blood-pressure(1).

In 1914 Vincent and Cameron(1) showed that there was a rapid and considerable fall of blood-pressure when deep voluntary breathing occurred in the human subject, comparable to falls obtained with anæsthetised animals during forced respiration. Other workers have also indicated similar effects. Boothby(2) plotted a curve of the blood-pressure during and after forced breathing and showed a most interesting fall and slow recovery exactly comparable in form to some of our present tracings. Hill and Flack(3) described considerable falls of blood-pressure.

More recently, however, doubt has been cast upon such results by the work of Collier, Densham and Wells(4) who failed to secure any effects at all upon the blood-pressure in 60 p.c. of their subjects, and they have postulated the existence of a compensatory mechanism in the human subject as distinct from other animals.

Little work of this nature has been performed upon the human subject, and the small amount done in relation to animals has been mostly in the anæsthetised condition.

A thorough investigation therefore seems invited.

Methods.

The apparatus used has been already described in detail elsewhere(5). Briefly it consists of a specially constructed water manometer and glass float, with connections to a sphygmomanometer armlet bag. The pressure in the bag is raised to a point about 30 mm. Hg below the subject's

systolic pressure, and a continuous record of the variations in the blood-pressure is obtained on smoked paper. It is obviously essential that the subject's arm should be kept quiescent during the experiment, and in all our work care has been exercised to see that the arm is supported in a comfortable position.

In a series of experiments forty-one subjects have been examined, and in most of the cases the experiments have been repeated on different days with identical results. The subjects adopt the sitting posture and at a given signal breathe rapidly and deeply through the mouth. This is continued for thirty seconds, but never long enough to produce apnœa.

Statement of results.

Thirty-seven out of forty-one subjects examined in this series recorded a fall of blood-pressure during the period of hyper-respiration. The depth of the falls varied. This was probably due to a combination of several factors, *e.g.* some subjects breathed more deeply and more quickly than others.

The average depression of blood-pressure was equivalent to 25 mm. Hg, the maximum being 53 mm. Hg, and the minimum 8 mm. Hg. In all instances the falls were rapid in onset and developed maximum depth shortly after cessation of forced breathing (Fig. 1). The recovery process

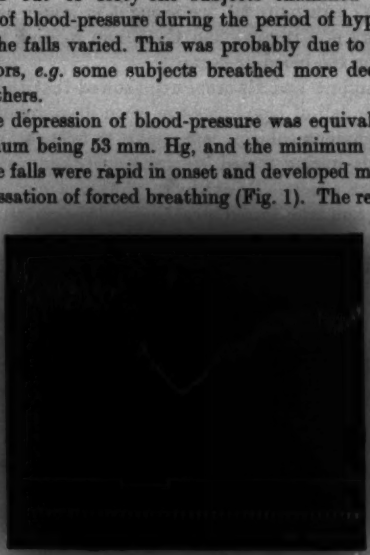

Fig. 1. Effect of forced breathing for 30 seconds. Fall of blood-pressure equivalent to 42 mm. Hg. All time tracings = 5 secs. intervals.

conformed to two distinct types—the rapid, and the gradual. In both types the recovery was complete.

Four subjects consistently produced rises of blood-pressure during forced voluntary breathing; the average rise was equivalent to 37 mm. Hg, the maximum 50 mm. Hg, and the minimum 18 mm. Hg.

All the rises of blood-pressure commenced simultaneously with the beginning of hyper-respiration, and then quickly returned to the mean pressure at the end of the experiment (Fig. 2).

Fig. 2. Effect of forced breathing for 30 seconds. Rise of blood-pressure equivalent to 50 mm. Hg. Note slow pulse rate.

Several variations of the experiment have been tried in an attempt to determine the causative factor or factors concerned in the differences of blood-pressure.

Different kinds of breathing. Instead of breathing rapidly and deeply as in the above series, the subjects were instructed to breathe rapidly and shallowly, slowly and deeply, and slowly and shallowly, the speed and depth of the respirations being varied over a considerable range. In the case of shallow breathing all subjects showed either no effect or a slight rise of blood-

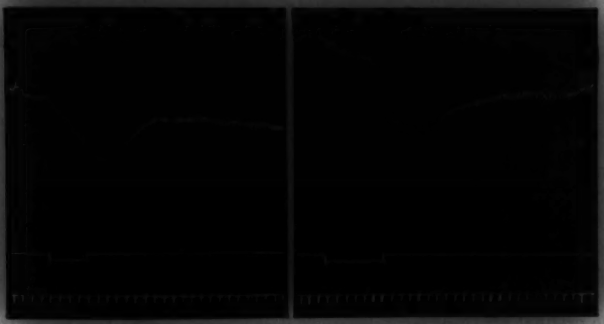

Fig. 3 *A*. Fig. 3 *B*.

Fig. 3 *A*. Fall of blood-pressure due to hyper-respiration for 20 seconds. Rapid recovery.

Fig. 3 *B*. Fall of blood-pressure due to hyper-respiration for 30 seconds. Slow recovery.

pressure. Otherwise, different kinds of breathing only affected the result quantitatively in both groups of individuals. The depressor effect was always most marked with rapid deep respiration.

Period of breathing. Lengthening the period of forced breathing markedly delayed the recovery process in the individuals normally giving quick recoveries (Figs. 3 *A* and 3 *B*). This phenomenon occurred irrespective of the type of breathing or the posture adopted.

Posture. A long series of experiments done with several individuals of both types in different postures showed clearly that posture makes a considerable difference to the results. In subjects recording falls of blood-pressure the falls were most marked in the standing position and least marked in the recumbent (Figs. 4 *A* and 4 *B*), whilst in subjects pro-

Fig. 4 *A*. Fig. 4 *B*.

Fig. 4 *A*. Subject in standing position. Forced breathing for 30 seconds.
Fig. 4 *B*. Subject in recumbent position. Forced breathing for 30 seconds.

ducing rises of blood-pressure the rises were most marked in the recumbent and sitting positions and least marked in the standing position (Figs. 5 *A* and 5 *B*).

Frequency of respiration. If in any given subject the periods of hyperpnœa succeed each other by short intervals, there is a marked diminution in the depth of the fall. After a longer interval (five minutes) the original depth of fall returns. This is largely caused by the subject failing to breathe so quickly and deeply because of fatigue, since a diminution similarly occurs in rise subjects. But the decrease of the depressor effect is more marked than that of the pressor and therefore cannot be wholly attributed to fatigue.

Respiration of 5 *p.c.* CO_2. Instead of breathing air, subjects inspired a mixture of 5 p.c. CO_2 and O_2. The result in fall subjects was a diminu-

Fig. 5 *A*. Fig. 5 *B*.

Fig. 5 *A*. Subject in recumbent position. Forced breathing for 30 seconds.
Fig. 5 *B*. Subject in standing position. Forced breathing for 30 seconds.

tion of the fall and a very marked and prolonged after fall (Fig. 6). In all such cases, however, there was an initial fall during hyperpnœa. In rise subjects there was a distinct diminution of the rise.

Fig. 6. Effect of forced breathing of mixture of 5 p.c. CO_2 and O_2.
Note initial fall followed by marked after-fall.

Inspiration of air at different temperatures. We have noted on many occasions that subjects giving large falls of blood-pressure in cold weather give smaller ones in warm weather. Specific experiments have

proved that inspiration of warm air diminishes the extent of the fall of blood-pressure. Almeida and Ozorio(6) found similar effects in animals.

Pulse rate. By connecting a Marey's tambour with an armlet bag on the other arm, it was possible to count the pulse rate before, during and after the experiment. This was done many times on several individuals of each group, and the results were remarkably consistent. In every subject producing falls of blood-pressure during hyper-respiration we found that the pulse rate remained remarkably constant at the normal. This appertained to the whole of the period of forced breathing. *Immediately* after this period there was a rapid acceleration which occurred with dramatic suddenness, and persisted for approximately ten seconds, and was followed by a marked slowing until the rate became subnormal. Shortly after, it returned to the normal. We have not found a single exception among "fall" subjects to this series of events.

It may be stated here that the only difference apparent to us between the two groups of individuals we have examined was in their habitual initial pulse rate. In all cases in which rises of blood-pressure were obtained the subjects possessed very slow pulse rates, the highest being sixty-two per minute and the lowest forty-five per minute. The largest rises were obtained in the individual with the pulse rate of forty-five per minute. On the other hand the more rapid the pulse the greater the fall in the other group.

Examination of the pulse rate of subjects giving rises during an experiment showed quite different results. There was a well-marked increase in rate during hyperpnœa which was still further augmented for a short time after cessation of forced breathing, and this was followed by a gradual return to the normal. The contrast between the two groups is shown in graphic form in Fig. 7.

Discussion of results.

It is clear from our experiments that the large majority (90 p.c. in our series) of people show a fall of blood-pressure when they breathe deeply and quickly for a short time in a resting condition. The human subject, therefore, compares with other animals in this respect. The suggestion that because falls of blood-pressure are obtainable in anæsthetised animals and anæsthetised human beings, the phenomenon is related to the paralytic action of an anæsthetic upon a compensatory mechanism, is without foundation since all the work of Vincent and Cameron can be and has been repeated on decerebrate animals in which all

anæsthetic has been eliminated. Our results clearly demonstrate the same reaction in man.

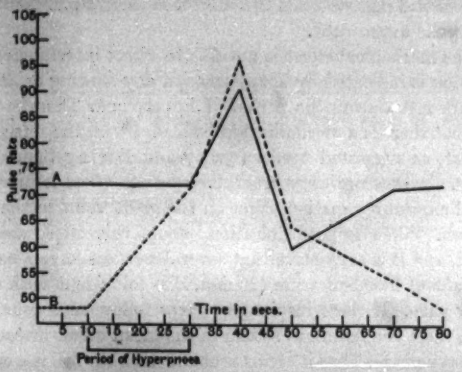

Fig. 7. Diagram showing difference in pulse rate at various stages of the experiment between a subject producing a fall of blood-pressure during forced breathing (*A*), and a subject producing a rise of blood-pressure (*B*). Whereas the pulse rate of *A* remained constant during hyperpnœa that of *B* rapidly accelerated. The recovery process is also different in each case.

We attribute the failure of some workers to obtain any effects upon the blood-pressure in man during hyperpnœa to the insensibility of their recording apparatus and an unsuitable type of breathing.

Our problem therefore resolved itself into an investigation as to why the blood-pressure fell and why in a small minority of individuals a pressor effect was always obtained.

Several theories have been advanced to explain the phenomenon in animals, and may be divided into two categories: (*a*) those which ascribe the fall in blood-pressure to a mechanical interference with the circulation in the thorax or with the heart's action (Vincent and Cameron(1), Janeway and Ewing(7) and others), and (*b*) those which regard the depressor effect as the result of deficiency of carbon dioxide in the blood caused by the hyper-respiration (Henderson(8) and others).

Hill and Flack(3) considered that the effects of deep breathing were largely due to interference with the circulation, their evidence being based on the different results obtained in different postures. In our own experiments we have been able to confirm such views and have demonstrated a constant quantitative variation of the blood-pressure according

to the posture of the subject—and this applies to both groups of individuals. In support of this mechanical theory is the fact that the depressor effect still persists when the subject inspires 5 p.c. carbon dioxide throughout the experiment.

That the mechanical effect is not due to direct interference with the heart's action is indicated by the absence of any slowing or acceleration of the heart rate during the period of hyperpnœa. Therefore it seems probable that there is a mechanical interference with the return of blood to the heart, as suggested by Janeway and Ewing(7) in their work on animals. In slow ordinary breathing no such effect is produced and the normal expiratory suction effect on the great veins in the thorax is in operation. When hyper-respiration occurs this effect seems to be neutralised, and it is suggestive that several workers have recorded considerable falls of blood-pressure (20 mm. Hg) coincident with each deep inspiratory gasp. If these inspiratory gasps follow each other in rapid succession, there is a failure to return to the mean blood-pressure during the expiratory phase. Thus it seems probable that the fall is a cumulative effect.

It is important to note, however, that there is a decided diminution in the fall when 5 p.c. carbon dioxide is breathed, and that by increasing the period of hyper-respiration the recovery process is delayed. This is evidence that the "washing out" of carbon dioxide from the lungs and consequent decrease in carbon dioxide tension of the blood is an additional (though subsidiary) factor in the production of the fall of blood-pressure.

The four subjects giving rises of blood-pressure all possessed an abnormally slow pulse rate, which markedly increased during the period of forced breathing in contradistinction to the pulse rate of the other group which did not vary during hyperpnœa. We consider that this is the cause of the difference between the two groups, the increased rate of the heart more than neutralising the mechanical and chemical effects.

Summary.

A determination of the effects of hyper-respiration in human beings has been made by means of an apparatus giving continuous records of variations in blood-pressure.

The large majority of subjects give a fall of blood-pressure which may be considered the normal reaction in man, since the small number of individuals giving rises have abnormally slow hearts, which fact accounts for the pressor effect.

Several variations of the basic experiment have been performed

which constitute evidence for the theory that the falls of blood-pressure are due to mechanical interference with the return of blood to the heart, and the lowered carbon dioxide tension of the blood as a subsidiary factor.

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NERVE FIBRES IN THE PITUITARY OF A RABBIT.

By MARGARET M. CROLL.

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Comparatively little is definitely known about the nerves in the three different parts of the pituitary. It is indeed stated by Bailey(1) that there is no proof of the existence of any other than sympathetic fibres to the blood vessels.

Rival theories are held as to the origin of such conditions as diabetes insipidus: (1) that it is due to an affection of the pituitary, either of the pars intermedia or pars nervosa, (2) that the pituitary plays no part in the production of symptoms, the condition being caused by lesions in the nervous system, more especially in the tuber cinereum. It seemed therefore of interest to ascertain if there is any innervation of the pituitary other than that to the vessels.

Berkley(3) was probably the first to use special methods for the demonstration of nervous elements in the pituitary. He worked with dogs and mice, and used chiefly the chrome-silver method of Golgi. He described in the posterior lobe both nerve cells and nerve fibres, and in the anterior lobe sympathetic fibres ending in knobs between the cells. It seems probable that the cells described in the posterior lobe by Berkley are really neuroglia. The present writer finds that they can easily be demonstrated in the human pituitary and in that of the rabbit by the Bielschowsky silver method, and that they resemble neuroglia more closely than nerve cells.

Herring(5), who discusses in some detail the views of the older anatomists on the subject, was not able to satisfy himself as to the nervous character of the cells and fibres in the pars nervosa, and considers them to be neuroglia. In the pars anterior of the cat's pituitary he did not succeed in demonstrating nerve fibres either by the Cajal or the Golgi methods.

Dandy(4) studied the anatomical but not the histological distribution of nerves. He states that the supply to the posterior lobe in the dog and kitten is poor in comparison with that of the anterior, and also that it is often possible to trace a single nerve fibre with its branches passing down the sheath and spreading out over the pars intermedia. He regards the nerves as secretory in function rather than vasomotor.

Tello(8) traced fibres from the pars nervosa passing into the pars intermedia and ending in the epithelium.

Pines(6), using a modification of the Cajal silver method, found that all parts of the pituitary—anterior, intermedia and nervosa—were richly innervated.

The whole question is still regarded by most people as unsettled.

Methods.

A number of methods were tried—many of them being entirely unsuccessful. In view of the capricious nature of both silver and gold methods it obviously may be fallacious to draw definite conclusions from negative results. A method, such, for example, as Löwit's, gives beautiful preparations with muscle, but is totally unsuited for the pituitary, as the latter dissolves in strong formic acid and is considerably damaged by weak.

As it was thought that the nerve supply to the pituitary would be mainly unmyelinated, it was decided to try various modifications of Ranson's silver-pyridine technique. The use of pyridine renders the staining of unmyelinated fibres more intense(7).

The following methods were used:

(1) Golgi methods.
   (*a*) Rapid modification.
   (*b*) Cox-Golgi modification.

(2) Sihler's method, used on frozen sections.

(3) Gold chloride.
   (*a*) The pituitary was placed in 2 p.c. gold chloride (brown variety of Merck) for 4 hr. One half was reduced by ultra-violet rays and one half by sunlight, the pieces being placed in water acidulated with acetic acid.
   (*b*) Löwit's method.

Of these three methods those of Golgi and Sihler were unsuccessful, while the gold chloride method gave deep purple sections in which no nerve fibres could be distinguished.

(4) Bielschowsky's silver method.

This proved unreliable, as connective tissue, blood and cell nuclei were stained. Comparing the sections later with successful specimens by the Ranson method, black nerve fibres could be identified in the pars intermedia. This method gave beautiful pictures of neuroglia in the posterior lobe.

(5) Modifications of Ranson's method.
  (*a*) Variations in the time in silver nitrate.
  (*b*) Variations in the time in pyrogallic acid.
  (*c*) Alterations in the fixing fluid.
    (i) The addition of 2 p.c. veronal in alcohol for 24 hr. instead of ammonia alcohol for 48 hr.
    (ii) 10 p.c. aqueous chloral hydrate for 24 hr. before using ammonia alcohol.
  (*d*) Alteration in the method of fixation by injecting the fixing fluid into the circulation of the animal before fixing the pituitary.

Ranson's method never failed to give results with the pars intermedia, but it seemed possible that negative results in the pars anterior might be due to failure of penetration either of the silver nitrate or of the fixing fluid, as the cells are very closely packed together. In all cases the pituitaries were cut in half before fixing. To test if a longer time in silver were necessary, pituitaries were left in the silver solution for periods varying from three to nineteen days. In no case did the increased time in silver appear to improve the results. Lengthening the time in the reducing fluid also seemed to have no effect. In the case of two other modifications, one, in which veronal in absolute alcohol was used for fixing, gave very poor results; the other, in which chloral hydrate was used before ammonia alcohol, was more successful, several sections showing black fibres in the pars anterior.

The most successful of the methods used was as follows: the blood vessels of a rabbit were washed out with Ringer's solution, which was injected into the aorta after all the lower vessels had been tied off. 160 c.c. of ammonia alcohol (100 p.c. alcohol with 1 p.c. ammonia) were then injected. The pituitary was removed, and cut in half; the anterior lobe was cut through again and the pieces fixed for 24 hr. in ammonia alcohol. After washing in distilled water, they were transferred to pyridine for 24 hr. and washed for another 24 hr. in many changes of distilled water. The pieces were then placed in the dark in a large quantity of 2 p.c. silver nitrate at 35° C. for seven days, rinsed in distilled water and reduced for 60 hr. in a 4 p.c. solution of pyrogallic acid in 5 p.c. formalin. They were washed, taken quickly through the alcohols, embedded rapidly in paraffin and cut at 18μ and in one or two cases counter-stained with safranin or methylene blue. This modification gave extremely good results in the case of the pars intermedia, and in some cases parts of the anterior lobe also showed black fibres.

A pituitary in which the animal had been injected with ammonia alcohol with 10 p.c. paraldehyde before using Ranson's method, gave sections which were much too dark to distinguish anything with certainty.

It is of course possible that some of the modifications would have proved successful had more variations of them been tried.

The pars intermedia is richly supplied with small non-myelinated nerve fibres, which often end in knob-like structures in the cells. These fibres are especially numerous at the junction of the intermedia and nervosa (Fig. 1), but are also found to spread all through the intermedia

Fig. 1. Pars nervosa and pars intermedia (rabbit). ×500, reduced ⅓. A modification of Ranson's silver-pyridine technique. Non-myelinated nerve fibres seen as thin black lines at the junction of the two lobes and extending into the intermedia. One fibre can be seen ending in a knob, apparently on a cell.

Fig. 2. Pars intermedia of rabbit. ×500, reduced ⅓. A modification of Ranson's silver-pyridine technique, counter-stained with safranin. Non-myelinated fibres seen as black lines.

towards the anterior lobe (Fig. 2). In deciding if these are simply vasomotor fibres, it is interesting to note that in the rabbit, while the pars anterior where nerve fibres are rare is extremely vascular, in the intermedia itself where there are numerous nerve fibres there are very few capillaries to be seen (Fig. 3). It would seem unlikely that the fibres are vasomotor in character.

Fig. 3. Pituitary of rabbit showing portions of the three lobes. × 65, reduced ⅓. Injected carmine-gelatine. The pars intermedia is comparatively non-vascular.

The possibility that these fibres are part of the connective tissues has been considered; but it does not seem to carry much weight. On comparing sections showing these fibres with others stained by Van Gieson or by Benian's method(3), it is obvious that the connective tissue is small in amount and does not resemble either in position or form the black fibres stained by Ranson's method. This method is considered to be specific for non-myelinated nerves, staining them black, whereas it usually stains connective tissue brown, if at all.

In the case of the pars anterior it is more difficult to be definite. Black fibres, running between the cells, and ending occasionally in small black knobs, are seen in parts of the sections (Fig. 4). That they do not occur constantly in all parts of the anterior lobe may be due to the capricious nature of the silver methods and to the resistance offered by the closely packed cells of this region of the pituitary. From the sinus-like nature of the blood vessels, it is hardly probable that any nerve fibres which are present are vasomotor.

The picture given by the posterior lobe is somewhat obscured by the number of neuroglial fibres. Black nerve endings in the walls of the

blood vessels resembling sympathetic endings are clearly shown and there are also thin black fibres extending for some distance round the vessels. These are perhaps rather numerous to be connected only with

Fig. 4. Pars anterior of rabbit. ×500, reduced ⅔. A modification of Ranson's method. The cells are out of focus in order to enable a long length of fibre to be seen.

the vessels. Fibres resembling those seen in the intermedia are seen near the junction of the two and often pass into the intermedia from the nervosa.

Some attempts have been made to demonstrate the nerve supply in the human pituitary. Perhaps owing to the difficulty of obtaining human material in sufficiently fresh condition and to the larger size of the human pituitary, the methods have not so far been successful. The work will be continued later.

Summary.

(1) Modifications of Ranson's silver-pyridine technique are most successful for demonstrating nerve fibres in the pituitary.

(2) The pars intermedia shows numerous thin black fibres running between the cells and sometimes ending in knobs. They are considered to be non-myelinated nerve fibres which are not vasomotor in function, as the intermedia in the rabbit is very poorly supplied with blood vessels. They do not resemble connective tissue, and appear constantly in sections stained by Ranson's method.

(3) Non-medullated nerve fibres are present in the pars anterior. They do not occur in such number nor so regularly as in the intermedia.

(4) Owing to the great wealth of neuroglial fibres in the pars nervosa the identification of nerve fibres in many areas is difficult. They are, however, abundant in the vicinity of the vessels and at the junction of the nervosa and intermedia.

The writer wishes to express her gratitude to Dr E. C. Eaves, at whose suggestion the work was carried out, for her help and interest. She thanks Mr R. Stewart for assistance with photo-micrography.

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CIRCULATORY AND RESPIRATORY CHANGES IN RESPONSE TO MUSCULAR EXERCISE IN MAN.

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Introduction.

It is well known that if muscular exercise is undertaken at a steady rate of working, some appreciable time elapses before the resultant increases in circulation and respiration attain an approximate equilibrium corresponding to the rise in metabolism. It would seem, therefore, not unlikely that the early stages of muscular work must be performed at a considerable disadvantage owing to this lag in the responses of both systems, even though the ultimate level attained by the circulation and respiration is sufficient to allow the work to be maintained at a steady rate almost indefinitely.

Thus, for example, the initial accumulation of lactic acid in the body might be considerably influenced by the rapidity with which the circulation and respiration respond to the muscular exertion, and if so, it ought to be possible to obtain evidence of this effect either during or after a short period of steady effort. Recently Jervell(1) has found that the lactic acid content of the venous blood in man rises most rapidly in the first few minutes of steady work, and suggests that this is largely due to the fact that the circulation rate is less at the start of exercise than later on.

With a view to testing this hypothesis, a number of pairs of experiments were performed. In each of these pairs the maximum rate of work was identical, but in the one case the subject started from rest with the maximum load, while in the other case the load was increased in several small increments to maximum, with the idea that the accommodation of circulation and respiration would be approaching completion by the time the maximum load was actually applied. Indirect evidence of the relative extents to which lactic acid accumulated was sought by following the course of the total respiratory exchange during preliminary rest and subsequent work, in some experiments; and the changes in the alveolar carbon dioxide percentage after stopping work,

in others. In spite of variety in the maximum load and in the type and duration of the preliminary grading chosen for different experiments, no definite evidence could be found by either of these methods that gradual approach to a full rate of working offered any advantage.

In view of this negative evidence it was felt desirable, before proceeding further, to obtain detailed information regarding the rate at which the circulation and respiration respond during the earliest stages of work in the subjects of the foregoing experiments, in the hope that this might throw light on the results which had been obtained. These detailed observations, made upon two subjects, C. G. D. and W. D. P., form the topic of the present paper. Table I shows for comparison some particulars regarding these subjects, as well as their respiratory exchange while at rest and when working on the bicycle ergometer at the rates of work which were usually employed.

TABLE I.

	C. G. D.	W. D. P.
Height	5 ft. 10 in.	5 ft. 8½ in.
Weight	10 st. 4 lbs.	11 st. 1 lb.
Age	45	26
Build	Light	Heavy
Condition	Completely out of training for strenuous exercise	In sufficiently good training to play football

	Total respiratory exchange					
	c.c. per minute at S.T.P.			c.c. per minute at S.T.P.		
	CO_2	O_2	R.Q.	CO_2	O_2	R.Q.
Rest	230	280	0·80	238	282	0·85
Pedalling "no load"	370	440	0·84	507	555	0·91
630 kg.m./min. work	1430	1530	0·94	1420	1630	0·87
840 kg.m./min. work	2000	1950	1·0	1818	1813	1·0

In correspondence with their respective physical conditions W. D. P. was found to be able to maintain steadily a higher rate of work than could C. G. D. (see especially Exps. 5 and 6, Fig. 3), and typical differences between other physiological responses are described throughout the text.

EXPERIMENTAL METHODS.

The muscular exercise was performed on a stationary bicycle ergometer throughout the investigations. In the earlier experiments a Martin ergometer (2) was used, but for reasons described later most of the experiments were done with a Krogh electric brake ergometer (3). A metronome was employed at first to give the rate for pedalling, but as a standard rate of 60 revolutions of the pedal wheel per minute was

adopted eventually, an electric bell was connected to a Brodie clock controlling a seconds time-marker. While the subject was working against the brake a current of air was directed on to him by an electric fan.

Pulmonary ventilation was recorded quantitatively on a smoked drum by the spirometer described by Haldane, Meakins and Priestley(4). Pulse rate was obtained from a pulse tracing recorded on the same drum by the use of a Marey tambour connected to a Mackenzie wrist tambour apparatus. The best position of the arm, as found by many tests, was partial flexion at the elbow with the forearm lying on an inclined plane and the elbow supported on a cushioned platform, which was set up at a convenient level close to the ergometer, and protected from vibrations.

Maximal systolic blood-pressure values were ascertained from the tracing given on the same drum as a pulse record by a recording U-tube mercury manometer, connected to a 12 cm. broad pneumatic armlet surrounding the upper arm of the subject. Both the armlet and the manometer were connected to a reservoir of air, under a pressure of about 25 cm. Hg, by means of an inlet tube controlled by a strong spring clip, and the junction was also fitted with a capillary glass outlet closed by another spring clip. By alternate manipulation of these clips it was easy to raise the armlet pressure rapidly to the desired level and then to allow it to fall at a suitable rate, making it possible to repeat complete observations as often as every 10 seconds but usually every 20 seconds, though when diastolic pressure records were taken in addition only two readings of each pressure per minute could be obtained. The reappearance in the pulse tracing of the first wave after complete obliteration was taken as the index of systolic pressure, and was found during rest at least to coincide with the "first sound" heard in auscultation of the brachial artery just below the armlet. In order to identify this reappearance sufficiently clearly to obtain an accurate index on each occasion it was found essential to avoid all but the smallest movements of the forearm causing secondary vibrations in the recording tambour. As it became increasingly difficult to obviate this imperfection as the work became harder, most of the experiments have been limited to a rate of work not above 840 kg.m. per minute to ensure sufficient and consistent accuracy.

Minimal diastolic blood-pressure was approximately determined in the first instance by using a small aneroid manometer ("Tychos") in a shunt from the pressure tubing between the armlet and the mercury

manometer. The index of diastolic pressure was taken as the usually definite decrease in amplitude immediately following maximum oscillation of the needle, and the moment when this appeared was recorded on the drum by a hand-operated electric signal, so that a pressure reading could be obtained from the mercury manometer trace superior in several ways to a visual estimate from the aneroid. As this method did not seem altogether satisfactory Pachon's oscillometer and Erlanger's sphygmomanometer were taken into careful consideration and tested in the hope that a better criterion of diastolic pressure might be obtained. However, as in the case of the standard auscultatory method also, these oscillatory methods were found to be even less easy to apply and interpret during muscular exertion, so eventually a simple recording oscillometer, based on Pachon's principle(5), was designed. With this new apparatus it was possible to obtain a *continuous* record, on the same drum as was used for systolic pressure and pulse rate, from which diastolic pressure indices could be secured more accurately and rapidly than previously. A diagram of the instrument is shown in Fig. 1.

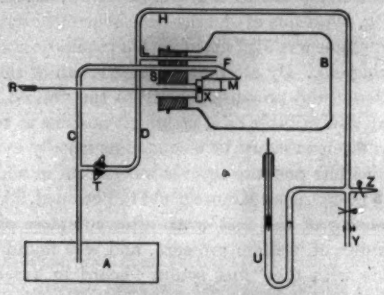


Fig. 1. Diagram of recording oscillometer.

B represents a stout-walled glass bottle of about 600 c.c. capacity acting as a transparent pressure chamber, which is closed by a large rubber stopper *S*, through which pass three glass tubes. *M* is an elastic membrane made of dental rubber dam 2 cm. in diameter and attached to the rim of a small glass funnel *F*, leading directly from the armlet *A via* as short a length of rubber pressure tubing, *C*, as possible. *R* is the writing pen of a light aluminium lever pivoted in a small spindle *X*, lubricated with thin vaseline in a bearing which is carefully ground in a brass plate firmly cemented to the inner end of a wide glass tube passing through the

stopper. The particular arrangement of this lever was designed to overcome the difficulty of accurately transmitting the movements of the thin rubber membrane from the interior of the pressure chamber to the outside without any leakage of air. It will be evident from the diagram that the membrane operates not only in an atmosphere always subjected to a higher pressure than that of the air outside the apparatus, but that this pressure alters considerably in correspondence with changes in armlet pressure. Tests proved that no appreciable leak of air occurred even at 300 mm. Hg pressure and that the lubrication of the pivot was sufficiently good to allow of small variations in the armlet pressure oscillations to be recorded very sensitively. *D* is a shunt airway from the armlet with an adjustable tap, *T*, near its connection with airway *C* and opening by a glass T-tube *L*, into *B*, and also by a longer tube *H* to the mercury manometer *U* with its associated compressed air inlet *Y* and outlet *Z*. As in Pachon's oscillometer the average pressure in the armlet and on both sides of the membrane must be equal at any pressure level if tap *T* be open, even during a change in pressure throughout the whole system. Closure of tap *T* at any steady average pressure not too far above the systolic value of the blood-pressure being investigated, results in oscillations in pressure from the armlet (due to alterations in calibre of the underlying brachial artery) being transmitted entirely to *M* and therefore indicated by *R*.

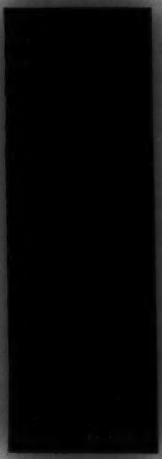
Before the extent of the oscillations, often called the "oscillation range," can be determined for any other armlet pressure, tap *T* has to be opened so as to allow renewed equalisation of average pressure on both sides of the membrane. It was discovered by experiment that if tap *T* was slightly opened and a high average pressure allowed to fall sufficiently slowly, that the membrane remained motionless until about 30 mm. Hg or so above the systolic pressure level, when there began to appear a series of oscillations rising from an almost straight horizontal base-line. As the pressure continued to fall these oscillations steadily increased in amplitude to a definite maximum after which they showed a rapid decrease in amplitude followed by a slower diminution. Tap *T* evidently functions as a selective leak allowing a continuous slow escape of air from the armlet and inner side of membrane, but effectually resisting the passage of all but a small fraction of the rapid air pressure fluctuations set up in the armlet by the underlying pulsation of the brachial artery. By repeated comparison of this continuous record or "oscillogram" with the auscultatory method, it was found during rest at least that the index of diastolic pressure as estimated by "change of

sound" ("point 4" between the third and fourth phases of Ettinger(6)) occurs very soon after the last maximal oscillation, i.e. during the usual short phase of rapid decrease in the oscillations. The diastolic pressure index in this investigation has therefore been taken as the point in the oscillogram at which the oscillations begin definitely to diminish after reaching a maximum. After varnishing the tracings the blood-pressure and other values were ascertained by drawing fine vertical lines through the appropriate points with a sharp instrument.

It was found impossible to record blood-pressure as well as pulmonary ventilation and pulse rate on the same drum owing to the limited width of paper. Accordingly one series of records was confined to pulmonary ventilation and pulse rate, while the other, which was taken under identical conditions, recorded pulse rate and blood-pressures, the record of pulse rate being common to both tracings. Typical specimens of the two types of record taken are shown in Fig. 2. *A* shows a continuous spirometer record uppermost, below this a pulse tracing, then a time-marker record of seconds, and lowest an electric signal trace denoting the start of work. *B* shows a record of pulse tracing uppermost, then time-marker and signal records, a manometer record of armlet pressure, below this an oscillogram showing the index of diastolic pressure, and lowest of all the manometer zero pressure line. In this portion of record taken during rest the breath was held subsequent to a short period of deep breathing so that the fundamental outline of the oscillogram would be evident. The series of records in *C* show in addition the vertical lines, corrected for zero errors, drawn from their respective pressure indices, from which it can be seen that the readings for both pressure values can be obtained about every 30 seconds or so.

Respiration even during rest produced a periodic variation in the amplitude of the oscillations derived from the brachial artery as shown by a small decrease over several beats during inspiration followed by a somewhat slower increase during expiration, superposed on the general outline of the oscillogram and most clearly shown with a steady average pressure or a very slow pressure fall. During work this variation became more marked, but as the pulse rate had increased it was possible to select the diastolic pressure index at the same phase of respiration on each occasion. The systolic pressure readings derived from the radial pulse index were necessarily taken at random, but their consistency showed that respiratory variation, though just detectable in the records, was unimportant, as might have been expected with such a small artery.

A



B

C

Fig. 2. Reproductions of typical tracings.

Experimental results.

While many observations on pulmonary ventilation, pulse rate and blood-pressure changes have been made after exercise of different kinds, few continuous records during the transition from rest to work and from work to rest have been published. Krogh and Lindhard(7,8), however, have produced much evidence regarding the nature of the respiratory and circulatory changes during both transitions; while Bowen(9) has charted and described the general alterations in pulse rate and systolic pressure during a period of cycling exercise lasting about half an hour and for about the same time thereafter. Masing(10), Lowsley(11), Barringer(12), Gillespie, Gibson and Murray(13), Liljestrand and Zander(14) and others have made somewhat infrequent observations of pulse rate and of one or both blood-pressures in man during work on some form of stationary ergometer. McCurdy(15) obtained high values, over 200 mm. Hg for systolic pressure during maximum effort in weight lifting as early as 1901, and was one of the first to emphasise the need for obtaining blood-pressure readings during work.

(*a*) *Pulmonary ventilation and pulse rate.* The first experiments described in this paper were made on pulmonary ventilation and pulse rate in the manner adopted by Krogh and Lindhard(7). The subject sat at rest on a Martin ergometer with his feet on the pedals and at the word of command started work with the full load on the brake. Typical examples of the results obtained are shown in the graphs of Exps. 1 and 3 in the upper portions of Figs. 3 and 4.

The abscissæ indicate time in minutes from the moment the work started while the ordinates show the pulse rate and pulmonary ventilation. The commencement of work also is indicated for clarity by a vertical line drawn from zero on the time scale.

In W. D. P. there is a very rapid rise in pulse rate, from 90 to 140 beats per minute by the end of 15 seconds, maintained during most of the working period. Pulmonary ventilation increases very rapidly within the same time interval, from 12 to 28 litres per minute, and then less rapidly till the end of the first minute after which it more slowly approaches a maximum level of about 40 litres per minute during the following two minutes. In C. G. D. also the pulse rate shows an initial but much smaller rise from the same resting value of 90 to 115 beats per minute in 15 seconds, with a distinctly gradual rise during the following 2¼ minutes towards an almost steady value of 140 beats per minute. Pulmonary ventilation rises rapidly within the first 15 seconds, from

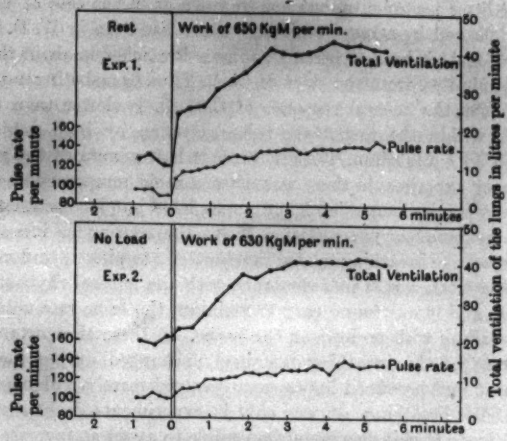


Fig. 3. C. G. D. Respiratory rate per minute 15.

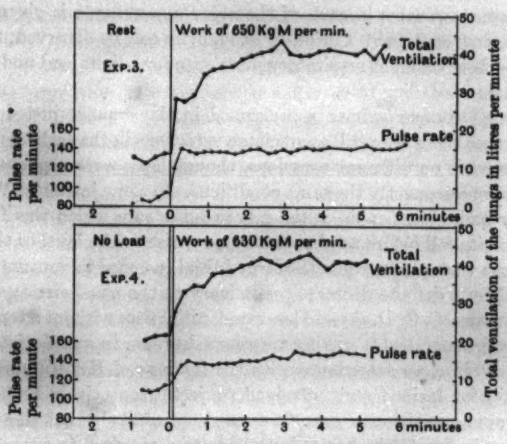


Fig. 4. W. D. P. Respiratory rate per minute 20.

12 to 24 litres per minute, but not so much as in the case of W. D. P. This is followed by a more rapid secondary rise than in W. D. P., but the same level of about 40 litres per minute is reached in about the same time, 2½ minutes, from the start of work. Thus from both records it is evident that the general response of C. G. D. is slower than that of W. D. P. within the first three minutes taken by both subjects to approach an equilibrium. Within the first half-minute of the work in preliminary experiments there was often a rapid temporary rise in the respiratory rate accompanied by an appreciable but inconstant fluctuation of the pulmonary ventilation above the continuous increase. In each subject the breathing tended involuntarily to pick up and maintain during the work a rate in accordance with the natural rhythm of the pedalling, and it was found easy to maintain the same rate voluntarily when pedalling with no load on the brake. In these circumstances the actual rate of the breathing remained unchanged during the entire experiment and increased hyperpnœa involved merely a change in the depth of the breathing. It was only in experiments dealing with the heaviest loads that it was found impossible to avoid an increase of rate of breathing as well as of depth. The striking changes in respiratory rate for heavy work are shown in Exps. 5 and 6, Fig. 5, but the particular constant rates in each of the other experiments is given in the legend below each pair. Curiously enough, as can be observed, the two subjects chose different respiratory rates even for a light load undertaken at the same pedalling rate.

A number of experiments performed in the manner just described showed that there were still appreciable variations in the earliest portions of the records on different occasions, though they were supposed to be obtained under exactly the same conditions. In some instances W. D. P. actually had a very rapid initial rise in pulse rate which was followed by a definite fall before a subsequent slower rise to the level of the early maximum took place; while the large initial increase in ventilation was followed by a definite decrease preliminary to the usual steady increase to maximum. C. G. D. showed less rapid initial rises without a temporary fall in pulse rate and a smaller transient decrease in ventilation. These results are in close accordance with the findings of Krogh and Lindhard(7,8) for trained and untrained subjects respectively undertaking work from a condition of rest. It seemed not unlikely that these effects were largely due to the heavy initial exertion required to overcome the inertia of the flywheel when it was necessary to raise its rate of revolution from zero to 175 per minute in the shortest time possible. Thus it became

clear that two uncertain factors were probably involved in the initial response of the respiration and circulation at the start of work undertaken from rest: (1) a nervous or psychic factor, (2) the sudden large momentary effort required to overcome the inertia of the flywheel.

With a view to avoiding this extra inertial factor and with the idea that the nervous or psychic factor might at the same time be reduced, it was decided to alter the method of applying the load. Instead of sitting at rest, the subject pedalled on the Krogh ergometer for several minutes with no load on the brake, and then the load was applied very rapidly by quickly adjusting the rheostat. The advantages of this method were (1) that the pedalling rate was maintained steadily throughout the entire experiment, (2) that the flywheel was already rotating at its proper speed when the load was applied, (3) that the subject required no verbal or other warning, but had merely to watch the metronome and maintain the steady pedalling rate, so that he had no idea of the precise moment when the load would be applied. While this method shows the changes which occur when the work done is considerably increased from a very low value, though the type and rate of muscular movement remain unaltered throughout, it does not afford an accurate picture of the changes which result on the transition from complete rest to considerable muscular exertion, and must therefore be carefully distinguished from Krogh and Lindhard's standard method. In actual practice the new method was found to be very satisfactory as repetition of experiments under circumstances intended to be identical effected the same responses, typical for the particular subject, on each occasion, so the method was adopted as a standard.

Exps. 2 and 4 graphed in the lower portions of Figs. 3 and 4 give examples of the results obtained in this way. These may be readily compared with the changes occurring on the transition from rest to the same rate of work. The distance between the lines in each vertical pair in this and subsequent diagrams represents the short time interval taken to apply or remove the load by rapid adjustment of the rheostat. It can be seen that the subjects still show typical differences between their initial responses. W. D. P. again exhibits the largest increase in ventilation at the very start, and pulse rate has increased rapidly enough within half a minute nearly to reach the maximum level attained. In the case of C. G. D. the pulmonary ventilation shows no sudden initial rise, but mounts fairly steadily and rapidly towards maximum, while pulse rate also increases gradually and reaches a nearly steady value in about 3 minutes. Similar but more marked changes occur with a rate

of 840 kg.m. per minute work as can be seen from Exps. 11 and 13 in Figs. 8 and 9 below.

In the case of very heavy work, 1260 kg.m. per minute, these typical differences become accentuated (see Exps. 5 and 6, Fig. 5). Under such

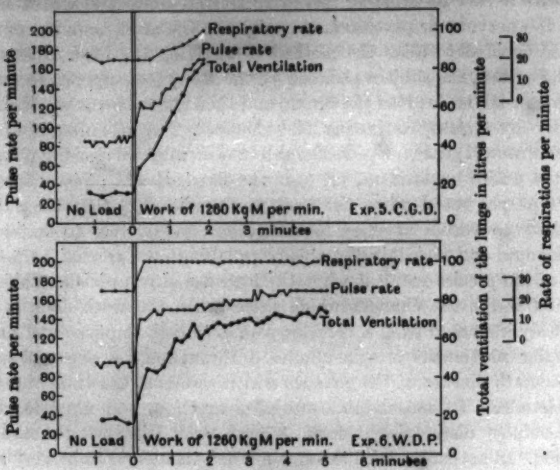


Fig. 5.

conditions for W. D. P. the initial rises in pulmonary ventilation, pulse and respiratory rates are very rapid. About 50 p.c. of the final increase in ventilation rate above "no-load" value is reached within 20 seconds after the start of work, while about 60 p.c. of the final increase in pulse rate is attained in 10 seconds, and thereafter there is a very gradual rise in both without a true equilibrium ever being struck. The respiratory rate actually rose from 15 per minute to the final steady value of 30 per minute in 18 seconds. C. G. D. showed a much less rapid initial response in all three values, but the rise continued steadily and fairly rapidly in each case until the hyperpnœa was so great that the experiment had to be stopped at the end of only two minutes' work, owing to an insufficient supply of air from the spirometer. In any case the subject could not have maintained this high rate of work much longer.

Pulmonary ventilation during recovery after moderate exertion, 630 kg.m. per minute, shows less striking differences between C. G. D.

and W. D. P. (see Exps. 7 *a* and 9 *a*, Figs. 6 and 7). The time intervals taken to return to equilibrium values are respectively 3 and 2 minutes, in close accordance with the periods required for the rise to maximum level at the start of work in each case. In addition the total deficit in ventilation below maximum during the early stage of the work period is almost as great as the total excess ventilation after the work is over. In the case of C. G. D. the pulse rate fell rapidly during the first minute and then very slowly thereafter, taking 5½ minutes to reach an equilibrium. W. D. P. showed an equally rapid preliminary fall with a distinct temporary rise during the second minute of recovery, soon followed by very steady values. A very similar comparison, but with even sharper contrasts for the complete sequence of events during work and recovery, can be drawn from Exps. 11 and 11 *a*, Fig. 8, for C. G. D., and Exps. 13 and 13 *a*, Fig. 9, for W. D. P., in which the rate of work was 840 kg.m. per minute (see below).

(*b*) *Blood-pressure and pulse rate.* The changes in blood-pressure due to quite moderate exertion are considerable, as can be seen from the graphs of Exps. 8 and 8 *a*, Fig. 6, and Exps. 10 and 10 *a*, Fig. 7, for 630 kg.m. per minute; also Exps. 12 and 12 *a*, Fig. 8, and Exps. 14 and 14 *a*, Fig. 9, for 840 kg.m. per minute.

During pedalling with no load the systolic pressure is higher for W. D. P. than for C. G. D., 134 mm. Hg as compared with 112 mm. Hg, but in both subjects the value finally obtained during effort is about the same for a given load. Whereas in the case of W. D. P. for 630 kg.m. per minute a value of 164 mm. is reached in 1½ minutes and thereafter there is very little increase in systolic pressure, C. G. D. takes 2½ minutes to attain 160 mm. after which the increase is likewise very gradual. With 840 kg.m. per minute the main rise to 170 mm. has occurred with 1 minute in W. D. P. and the gradual approach to the highest value of 180 mm. is not completed till after the third minute. In C. G. D. the rise in systolic pressure is gradual from the start though the final value of 200 mm. reached in 4 minutes is distinctly higher than that attained by W. D. P. in the same time. The diastolic pressure, as determined by the oscillometer, remained almost constant both during and after work of 630 kg.m. per minute in W. D. P., but there was an appreciable rise of about 20 mm. during the work followed by a corresponding fall after the load was removed in the case of C. G. D. With the heavier work of 840 kg.m. per minute W. D. P. also showed a distinct rise in diastolic pressure, which is apparent during the first minute after application of the load (see Exps. 14 and 14 *a*, Fig. 9). Unfortunately the corresponding

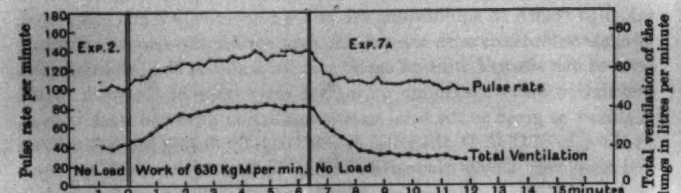


Fig. 6. C. G. D. Respiratory rate per minute 15.

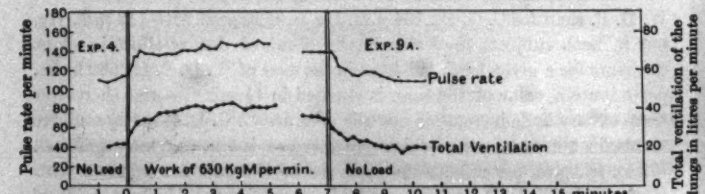


Fig. 7. W. D. P. Respiratory rate per minute 20.

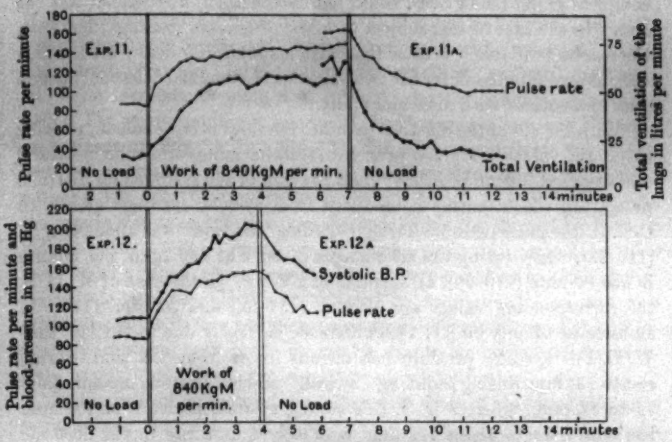


Fig. 8. C. G. D. Respiratory rate per minute 20.

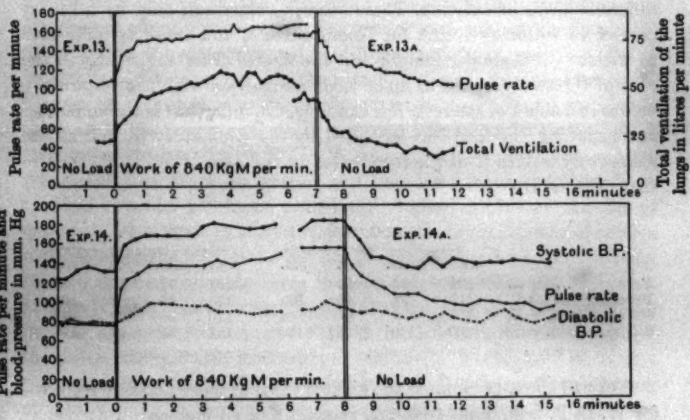


Fig. 9. W. D. P. Respiratory rate per minute 30.

complete graph for C. G. D. could not be obtained, as the load was too heavy in the case of this subject to allow of reliable readings; but from the results available it is clear that with a sufficiently heavy load, which was, however, different for the two subjects, the brachial diastolic pressure rises appreciably with muscular effort.

With the aid of the diastolic pressure readings it is, of course, possible to obtain values for pulse pressure (systolic minus diastolic pressure) and so-called "mean pressure" (half the sum of the systolic and diastolic pressures), which cannot be deduced from systolic readings alone. In C. G. D. the pulse pressure during pedalling "no load" was about 40 mm. (112–72), while during the later stages of work at 630 kg.m. per minute it was 80 mm. (170–90), an increase of 100 p.c. In the case of W. D. P. the corresponding values are 52 mm. (134–82) and 80 mm. (170–90), an increase of only 59 p.c. This difference is largely due to the fact that W. D. P.'s systolic pressure has already risen from 122 mm. during rest to 134 mm. during pedalling "no load" and his diastolic pressure from 72 to 82 mm., whereas C. G. D.'s resting systolic pressure of 104 mm. has only risen to about 112 mm. with only 4 mm. rise in the diastolic value.

Table II gives a summary of the average values of the circulatory data obtained from many experiments during rest and at the two different levels of exertion. These *average* values can only be taken as correct to within ± 4 mm. for blood-pressure, and those of pulse rate to within ± 3 beats per minute, but the figures differ sufficiently in the case of the two subjects to make their comparison with the respiratory values in Table I of interest. For example, the difference between resting and pedalling "no load" as indicated by the circulatory as well as the respiratory values is distinctly greater in the case of W. D. P. than in that of C. G. D.

TABLE II.

	C. G. D.				W. D. P.			
	S.B.P.	D.B.P.	P.P.	P.R.	S.B.P.	D.B.P.	P.P.	P.R.
Rest	104	68	36	85	122	72	50	85
Pedalling "no load"	112	72	40	100	134	82	52	105
630 kg.m./min. work	170	90	80	145	170	90	80	140
840 kg.m./min. work	200	[100]	[100]	150	182	98	84	150

P.P. = pulse pressure in mm. P.R. = pulse rate per minute.

For 840 kg.m. per minute the highest value of pulse pressure recorded for W. D. P. is 92 mm. (184–92), representing an increase of 84 p.c. as compared with pedalling "no load." Though no values of diastolic pressure were obtained from C. G. D. for this load it is reasonable to suppose

from the values for the lighter load that the rise was not above 100 mm. If this were so, then the corresponding value would be 104 mm. (204–100), equivalent to an increase of 250 p.c. compared with "no load" value. The "mean pressures" for C. G. D. during pedalling "no load" and during 630 kg.m. per minute work were 92 mm. and 130 mm., an increase of 41 p.c. The corresponding values for W. D. P. were 108 mm. and 130 mm., an increase of only 21 p.c., while for 840 kg.m. per minute the rise to 138 mm. represents an increase of 29 p.c., corresponding to a probable value of 152 mm. or an increase of 65 p.c. in the case of C. G. D. As the rise in diastolic pressure was inconsiderable for the lighter load, it will be evident that the change in "mean pressure" caused by such work is, as a rough approximation, but little more than half the change in systolic pressure. However, it must be emphasised here that this arithmetic mean of two pressures possessing different physical characteristics is empirical, and probably has no simple fundamental interpretation, particularly in relation to relative circulation rates.

The records of pulse rate taken along with those of the blood-pressures were almost identical with those secured during measurement of the pulmonary ventilation, though on different occasions the same subject showed small differences in the maximum value obtained for a given load. This close and consistent correspondence between the values of the common factor in the two series of experiments provided good evidence of equal physiological response for each subject during experiments which, though performed on different occasions, were intended to be identical. The changes in pulse rate follow much the same course as changes in ventilation and systolic pressure, and even more closely those of pulse pressure, C. G. D. showing the typically slower initial response as compared with W. D. P., whose pulse increases very rapidly within the first 20 or 30 seconds and then rises much more slowly to the final value for 630 kg.m. per minute. Indeed in C. G. D. the change is gradual from the very start and shows hardly any indication of sudden increase after the load is undertaken, though the pulse rate eventually reached is practically the same in both subjects. With 840 kg.m. per minute W. D. P. shows a very rapid initial rise with only a very gradual increase after the first minute. C. G. D. has a less rapid initial rise and the pulse rate was still increasing at the end of 5 minutes. W. D. P.'s pulse rose from 80 to 150 per minute; C. G. D.'s from 86 to 156 per minute. Owing to the limits of the apparatus individual records were restricted to a duration of about 9 minutes. It will be seen that during the working period a perfect equilibrium is not reached in the majority

of the experiments, though in the later stages of work the changes in pulmonary ventilation, pulse rate and systolic pressure are all very gradual. This phenomenon is considered below, in the discussion.

On removal of the load the ventilation, pulse rate and systolic pressure fall rapidly within the first minute and then more slowly towards the values obtained before the load was applied, while the diastolic pressure falls very little but perceptibly. The changes occurring in all four factors during this recovery period can be readily compared in Figs. 6 to 9 with those shown during application of the load, those in Fig. 9 providing the clearest contrasts.

Discussion of results.

When work was undertaken suddenly from rest, as in the earliest experiments, the results obtained were in close accordance with those described by Krogh and Lindhard(7) for similar conditions. These authors put forward the view that the rapid initial increase in pulmonary ventilation and pulse rate was caused by "irradiation of impulses from the motor cortex" rather than by reflex action, partly because one of their subjects showed a marked increase in ventilation when he anticipated the application of a heavy load but actually started pedalling without any brake load. They also described three phases in the ventilation changes, a sudden initial increase followed by an increasingly slow rise towards an approximate equilibrium, whose level depended on the extent of the change in metabolism.

The experiments described in this paper show that similar but less marked changes occur when, instead of starting from rest, the amount of work per minute is considerably increased from a very low value (that of pedalling "no load"), while the nature and rate of the muscular movement is maintained uniformly. These changes in pulmonary ventilation were pretty closely followed throughout by the simultaneous alterations in pulse rate and blood-pressure. It seems probable that the smaller initial responses shown when the work was undertaken in this new way were due to reduction of the psychic or nervous influences involved in starting from rest and elimination of the considerable initial effort required to overcome the inertia of the flywheel. Thus C. G. D. showed a gradual increase in ventilation and pulse rate from the very start of work when undertaking the lighter load while pedalling, in contrast with the very sharp rise induced by undertaking the same load from rest (see Exps. 1 and 2, Fig. 3). Even so, close inspection of

the spirometer records shows that the depth of the first or second inspiration after application of the load was usually greater than those immediately following though the respiratory rate was constant (see *A*, Fig. 2). This effect was even more marked in the case of W. D. P., though in both subjects much less obvious than in some of Krogh and Lindhard's experiments, as would be expected. Whatever the contributory causes in their experiments the effect seems more likely to be due to reflex rather than psychic causes in the standard type of experiment adopted in this investigation.

Another transitory and more variable effect was noticeable in some of the blood-pressure records obtained from C. G. D. and well shown in Exp. 8, Fig. 6. Systolic and sometimes also diastolic blood-pressure readings within the first minute of work either fell below, or scarcely increased above, the average "no load" value. This phenomenon was noticed by Gillespie, Gibson and Murray(13) who suggested that it might possibly be due to deepened inspiration. In view of the frequently repeated observation that systolic pressure always rose rapidly from the start even with a greater initial respiratory increase for W. D. P. as compared with C. G. D., it seems more probable that the cause of this delayed pressure rise is largely due to a less rapid general vasomotor response. In this connection Exps. 5 and 6, Fig. 5, are interesting in that they suggest the importance of the rapidity of the initial response of the circulation and respiration in relation to the capacity of a subject to maintain a high rate of work. As Krogh and Lindhard pointed out, unless the circulation and respiration were able to adapt themselves rapidly to the instantaneous and enormous rise in muscular metabolism coincident with sudden and strenuous exertion, before the vasomotor and respiratory centres were directly influenced by the more gradual increase in metabolites carried to them by the blood stream, then such muscular activity could not possibly be sustained for more than a fraction of a minute. The active muscles would be hopelessly asphyxiated before a fresh supply of oxygen could reach them, and the excess of lactic acid and carbon dioxide be disposed of.

When the records of systolic pressure and pulse rate are compared with those of pulmonary ventilation it can be seen that not only are the initial responses similar, but that the times taken to reach an approximate equilibrium are about the same for all three factors. Thus for 630 kg.m. per minute it takes about 3 minutes for these factors to approach equilibrium, while W. D. P. only requires about 1½ minutes. If one may fairly take the systolic, diastolic and pulse pressure changes

together as an index of circulatory alteration, it is evident that the accommodation of the circulation requires about the same time as that of the pulmonary ventilation to meet the needs imposed by the rise in metabolism, and that all this is largely effected within 3 or 4 minutes in the case of even the heavier load chosen for either subject.

With regard to the actual systolic pressure values attained during the moderate rates of work employed in these experiments, it seems advisable to point out that they are much higher than those suggested even very recently by Norris(16) and others for dumb-bell exercise above 1000 kg.m. per minute, partly on the basis of Cotton, Rapport and Lewis'(17) graphs obtained entirely after effort had ceased. On the other hand the high systolic pressure and pulse rate values obtained by Hill and Flack(23) in athletes and by Pembrey and Todd(24) in trained and untrained men very soon after running up and down stairs are very similar to those shown in the earliest portions of the recovery curves graphed in this paper. As their readings fall just as rapidly during rest immediately following exertion it seems reasonable to suppose that the values during the exertion were at least as high as those actually measured during the ergometer exercise. The results of these and other types of muscular exertion and even of identical exercise followed by rest must be carefully distinguished from the particular experiments cited in this paper. Also the fallacious assumptions involved in attempting to deduce blood-pressure values during exertion from readings taken even a fraction of a minute after removal of the load must now be apparent from the graphs in Figs. 6 to 9. The contrast between the rises in systolic pressure for different increments of work per minute relative to "no load" value was greater and more constant in each subject than that between the corresponding increases in pulse rate, which did not always start from the same "no load" value as did the pressure. This result is in accordance with the observations of other investigators(13,14,17,18) for a constant rate of limb movement with different loads. Unfortunately no values are available for similar amounts of work per minute performed at different pedalling rates for comparison of the relative efficiencies(18), and the corresponding effects on pulse rate and blood-pressure. The fact that pulse rate and pulmonary ventilation continued to increase slowly even several minutes after the main rises had occurred suggests that possibly there was a slight temperature effect, only making itself noticeable some time after the actual start of work, though it was hoped to minimise this complication by the measures described previously.

Neither blood-pressures nor pulse rate can in themselves afford a true index of the output of the heart, but many attempts have been made to deduce alteration in circulation rate from simultaneous changes in these factors. The most recent suggestion due to Liljestrand and Zander(14) is that changes in circulation rate can be inferred from the formula $\left[\frac{\text{P.P.} \times \text{P.R.}}{\text{M.P.}}\right]$, in which P.P. represents pulse pressure, P.R. pulse rate, and M.P. "mean pressure," in the brachial artery. Using this method of calculation it would appear that the relative figures for circulation rate in the case of C. G. D. are: at rest 100, pedalling "no load" 130, later stages of 630 kg.m. per minute work 270; while for W. D. P. the corresponding figures are 100, 111, 193, and 237 for 840 kg.m. per minute. However, as the universal validity of this and similar formulæ can by no means be regarded as definitely established, no attempt has been made in this paper to apply such a method of calculation in detail throughout the experiments. Indeed the relation of brachial artery pressure values, obtained by the armlet method, to aortic pressures may be even less direct during a period of muscular exertion than seems probable for the resting condition (see Dawson(25)). Though every care was taken to protect the upper limb carrying the armlet from strain of any sort, it cannot have been entirely immune from some increased reflex tone due to the considerable activity of muscles in other parts of the body, and may therefore have required extra armlet pressure for its necessary distortion by compression over the region of the brachial artery and underlying bone.

Perhaps more important might be the hypertonus, presumably induced in the whole arterial system of the upper limbs for compensatory restriction of blood flow in an inactive region of the body whose aortic supply had greatly increased in pressure as well as in output, and possibly extending from the smaller vessels so as to be appreciable even in the brachial artery. The only direct evidence obtained for the existence of such a hypertonus was similar to that described, for example by Stacey Wilson(19), for pathological hypertonus in subjects at rest, namely, a much more rapid approach to a more prolonged plateau in the "oscillation range" outline during work as compared with what was obtained previous to or sometime after the working period. Whatever the extent of these two types of increased tonus the total effect would be to make compression and distortion of the brachial artery require an armlet pressure greater than that necessitated by the lateral blood-pressure alone. Armlet pressure readings derived from quite critical indices would thus tend to be too high for estimations made while the

subject was working. Now the actual pressures obtained seem very high for an artery in an inactive limb and may not at all accurately represent even the relative increase in the lateral blood-pressure, as would be measured by a manometer and canula. It is interesting to note in passing that Russell(30) insisted on the importance of this discrepancy in cases of marked and often palpable hypertonus (as distinct from arterio-sclerosis) of the brachial artery in pathological conditions with subjects at rest, and it is at least probable that considerable muscular activity may produce a transient example of a similar phenomenon.

Lindhard(26), also Lythgoe and Pereira(21), have shown that the rate of O_2 intake falls much more rapidly than pulse rate after strenuous exercise, and suggested that circulation rate and therefore cardiac output per beat probably decrease more rapidly than pulse rate, at least in the earliest stages of recovery. However, the important factor of change in the percentage utilisation of oxygen, as described, for example, by Douglas and Haldane(22), must also be taken into account during most of a large and rapid alteration in circulation and respiration. In any case, from the close harmony which has been shown to exist in the experiments described between the changes in ventilation pulse rate and blood-pressure, both at the start and end of undertaking a steady rate of work per minute, it is reasonable to suppose that the respiration and circulation adapt themselves to muscular activity at all stages in a closely coordinated fashion. Moreover, this coordination shows a characteristic difference between different individuals in accordance with their respective physical conditions.

Conclusions.

1. Observations have been made at frequent intervals on pulmonary ventilation, pulse rate and blood-pressure at the commencement and cessation of steady muscular work in two subjects of different age and physical training.

2. A simple form of recording oscillometer has been designed for determination of the diastolic blood-pressure.

3. The initial changes in respiration and circulation, which occur during the earlier stages of work at a steady rate, show variations depending on the way in which the load is undertaken.

4. Comparison of the changes in pulmonary ventilation, pulse rate and blood-pressure suggests that the rate of accommodation of the cir-

culation follows a very similar course to that of the respiration when the metabolism is altered on account of change in muscular activity.

5. The rises in total ventilation, systolic and pulse pressures and to a lesser extent pulse rate were roughly proportional to the relative increments in the amount of work per minute, and therefore to the corresponding metabolic increases.

6. The times taken by all factors to attain an approximate equilibrium were dependent not only on the load but on the particular subject.

7. There is a definite difference between all the corresponding responses of the two subjects in accordance with their respective physical conditions.

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ACTION OF CALCIUM IONS ON THE FROG'S HEART.

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The process of blood coagulation has been shown by two of us(1) to depend on the non-diffusible calcium in the blood and not upon the ionic calcium. The addition of citrate in sufficient quantity to reduce the ionic calcium actually increased the coagulability of the blood, and the coagulation was inhibited only when sufficient citrate was added to inactivate the non-diffusible calcium. The present investigation was undertaken to determine the cause of the paralysis of the frog's heart that is produced by calcium deprivation, or by addition of citrate.

Lack of calcium arrests the frog's heart in a few seconds (De(2)), but a heart, after perfusion with calcium-free Ringer for a considerable time, can be restored to almost normal activity very quickly by perfusion with normal Ringer (Arima(3), De(2)). These facts make it highly probable that the paralysis of the heart produced by lack of calcium is not due to removal of calcium from the interior of the heart cells, and indicate that the action is produced at the cell surface.

Methods.

The normal Ringer's fluid had the percentage composition: NaCl 0·65, KCl 0·015, $CaCl_2$ 0·012 (approximately 1 millimolar), NaH_2PO_4 and Na_2HPO_4 0·05, *p*H 7·5. In addition to this mixture (phosphate buffer) we used the same fluid without phosphate, and brought to *p*H 7·5 by addition of *N*/100 NaOH (no buffer). This latter mixture was made up with distilled water that had been exposed to air and therefore contained a certain amount of carbon dioxide. When the concentration of calcium was raised, or when sodium citrate was added, the sodium chloride content was reduced to maintain the osmotic pressure constant.

The concentration of calcium ions was determined by the method of Brinkman and van Dam(4). The response of the frog's ventricle was measured isometrically by a method described by one of us(5). The

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pressure added on systolic contraction was taken as the measure of the heart response. During long experiments the general condition of the frog's heart declined; this source of error was eliminated as far as possible by taking frequent measurements of the response with normal Ringer's fluid and expressing our measurements as percentages of the most recent normal response.

Effect of variations in calcium content.

The amount of ionisation, with varying concentrations of calcium chloride, in the two mixtures described above, was as follows:

Table I.

Effect of varying the calcium concentration on the concentration of ionic calcium.

	Millimolar concentration of $CaCl_2$					
	0·25	0·5	1·0	2·0	4·0	8·0
	Millimolar concentration of ionic calcium					
(i) No buffer	0·223	0·35	0·675	1·08	1·8	3·6
(ii) Phosphate buffer	—	0·21	0·48	0·68	—	—

These figures show that even when no buffer is present the calcium chloride is only partially dissociated, and that the presence of phosphate buffer produces a further reduction in the concentration of calcium ions.

The effects of variation of calcium content on the frog's heart were as follows:

Table II.

Effect of varying the calcium concentration on the response of the frog's heart.

	Millimolar concentration of $CaCl_2$						
	0·25	0·5	1	2	4	8	16
	Added systolic pressure produced by frog's heart, expressed as p.c. of maximum response						
(i) No buffer	8	40	85	100	100	96	0
(ii) Phosphate buffer	15	45	80	100	94	92	0

A comparison of Tables I and II shows that the heart is tolerant of a large excess of either total calcium or calcium ions, but the results obtained when the total calcium content was below normal could be explained equally well by assuming that the heart was affected by the total concentration of calcium, or by the concentration of calcium ions present.

The effect of citrate.

The addition of citrate to Ringer's fluid containing no buffer reduced the ionic concentration of calcium in the manner shown in Table III.

Table III.

Millimolar concentrations of calcium ions with varying concentrations of calcium chloride and of sodium citrate. (No buffer. *p*H 7·5.)

	Millimolar concentration of sodium citrate									
	0	0·25	0·5	1	2	4	8	12	16	32
M.molar conc. of $CaCl_2$	Millimolar concentration of calcium ions									
0·25	0·225	—	—	—	—	—	—	—	—	—
0·5	0·35	—	—	—	—	—	—	—	—	—
1·0	0·675	0·31	0·215	0·195	0·108	—	—	—	—	—
2·0	1·08	—	0·31	0·24	0·195	0·09	—	—	—	—
4·0	1·8	—	—	—	0·4	0·307	0·155	0·108	—	—
8·0	3·6	—	—	—	—	0·4	0·307	—	0·155	0·110

Fig. 1 shows the effect of citrate in reducing the force of response of the frog's heart with concentrations of $CaCl_2$ ranging from 0·25 to 16

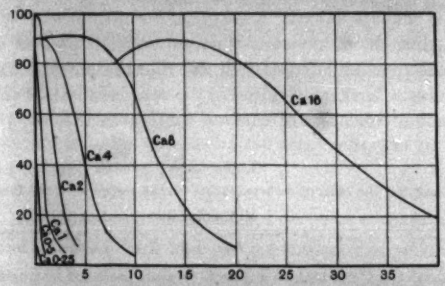

Fig. 1. Action of citrate on frog's heart in presence of calcium chloride varying from 0·25 to 16 millimolar. Ringer's fluid without buffer. *p*H 7·5. Ordinate: systolic pressure expressed as p.c. of maximum. Abscissa: millimolar concentration of sodium citrate.

millimolar. A comparison of Table III and Fig. 1 shows that the response of the frog's heart does not vary according to the concentration of calcium ions found in the solution. For example, in the absence of citrate, 0·5 millimolar calcium chloride, which gives 0·35 millimolar calcium ions, causes a heart beat 40 p.c. of the maximum. But when 0·5 millimolar citrate is added to 2 millimolar calcium chloride, the ionic calcium is reduced to 0·31 millimolar, and yet the force of response of the heart remains nearly 100 p.c. of the maximum.

In all cases when the ionic calcium was reduced by addition of citrate the effect on the frog's heart was much less than when the ionic calcium was reduced to an equal level by reduction in the total concentration of calcium chloride. The results in Fig. 1 cannot, however, be explained by supposing that the calcium in solution binds some of the citrate and the remainder of the citrate acts on the heart. This is shown by considering the mixtures that produce an equal reduction in the response of the heart. For instance, Fig. 1 shows that a reduction of the response of the heart to 40 p.c. of its maximum is produced by the following millimolar combinations of calcium and citrate: 0·5 and 0, 1 and 0·8, 2 and 2·5, 4 and 5, 8 and 17·5, and 16 and 31·5.

A series of experiments similar to those shown in Fig. 1 made with phosphate Ringer gave results which showed rather less divergence between the reduction produced by citrate in the ionic calcium and in the response of the frog's heart. The divergence was, however, still very marked.

In all these experiments we were of course comparing the response of the heart with and without citrate, with the ionic concentrations of calcium as measured in the fluid introduced into the heart. The behaviour of the heart is however determined by the fluid actually in contact with the heart cells, and since these cells are continually giving off carbon dioxide the fluid actually in contact with the heart cells must have a considerable carbon dioxide content and its probable reaction is nearer *p*H 7·0 than *p*H 7·5.

We therefore repeated our experiments using a solution containing 0·05 p.c. $NaHCO_3$ and adjusted with carbon dioxide to *p*H 7·0. This gave the following values for the ionic concentrations of calcium.

TABLE IV.

Millimolar concentrations of calcium ions with varying concentrations of calcium chloride and sodium citrate. 0·05 p.c. sodium bicarbonate. *p*H 7·0.

	Millimolar concentration of sodium citrate						
	0	0·5	1	2	4	8	12
M.molar conc. of $CaCl_2$	Millimolar concentration of calcium ions						
0·5	0·18	—	—	—	—	—	—
1·0	0·39	0·3	0·24	—	—	—	—
2·0	0·43	—	0·31	0·19	—	—	—
4·0	0·6	—	0·51	0·4	0·24	—	—
8·0	1·35	—	—	—	0·43	0·27	0·19

When the ionic calcium is below normal, the variations in the ionic concentrations in this series of observations agree quite well with the

variations in the response of the frog's heart as shown in Fig. 1. In the first place when no citrate is present a comparison of Table IV and Fig. 1 gives the following results:

TABLE V.

Millimolar calcium chloride concentration					
0·25	0·5	1·0	2·0	4·0	8·0
Relative response of frog's heart					
8	40	85	100	92	92
Absolute millimolar concentration of calcium ions					
—	0·18	0·39	0·43	0·6	1·35
Concentration of calcium ions expressed as p.c. of concentration at $CaCl_2$ = 2 millimolar					
—	42	91	100	140	315

The continuous lines in Fig. 2 show the concentrations of calcium ions with varying concentrations of calcium chloride and of citrate. The

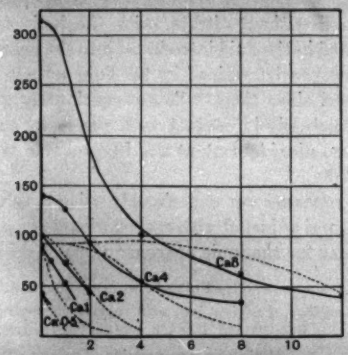

Fig. 2. Effect of sodium citrate on concentration of calcium ions, with concentrations of calcium chloride varying from 0·5 to 8 millimolar; Ringer's solution with carbonate at *p*H 7·0. Abscissæ = millimolar concentration of sodium citrate. Solid lines show millimolar concentrations of calcium ions expressed as the percentage of the concentration of calcium ions when $CaCl_2$ = 2 millimolar and no citrate is present. (Ca^{++} = 0·43 millimolar.) Dotted lines show responses of frog's heart expressed as percentages of the value which was obtained when $CaCl_2$ = 2 millimolar and citrate = 0.

results are taken from Table IV, and are plotted as percentages of 0·43 millimolar calcium ions. This particular value was taken as 100 because it appeared to be the optimum concentration of calcium ions for the

response of the frog's heart. The dotted lines in Fig. 2 are taken from Fig. 1 and show the response of the frog's heart. A comparison of the continuous and dotted lines in Fig. 2 shows in the first place that increase in the ionic calcium above 100 (*i.e.* 0·43 millimolar) does not increase the response of the frog's heart and indeed produces a slight depression: hence, when the ionic calcium is above 100 the two sets of curves diverge completely. Decrease in the ionic calcium below 100 in all instances is associated with reduction in the response of the heart. The extent of the reduction in the response of the heart is not exactly proportional to the extent of reduction of the ionic concentration below 100, but the curves do not show any consistent divergence. In considering these results it must be remembered that it is impossible to get very accurate figures either for the low concentrations of ionic calcium that are under consideration, or for the response of a frog's heart partially poisoned by calcium deprivation.

The extent of the agreement is perhaps best shown by considering the figures obtained for the response of the frog's heart, with a series of mixtures that contain equal concentrations of ionic calcium. For example, the following five mixtures of calcium and citrate contain 40 (*i.e.* 0·17 millimolar) calcium ions (the figures represent millimolar concentrations): 0·5 and 0, 1 and 1·3, 2 and 2·1, 4 and 5·7, 8 and 12. The response of the frog's heart with these five mixtures was as follows: 40, 23, 45, 30 and 40.

The effect on the response of the frog's heart of variations in the total quantity of calcium present and also the effect of addition of citrate can be interpreted as due to the variations in the concentration of calcium ions in the fluid in contact with the heart cells, provided that certain assumptions are made, and a considerable experimental error be admitted. On the other hand there is no obvious relation between the effect produced on the frog's heart and the amount of citrate uncombined with calcium that is present, nor could we find any relation between the effect produced on the frog's heart and the concentration of any undissociated calcium compound in the perfusion fluid.

The antagonism between calcium chloride and sodium citrate.

The concentrations of calcium chloride and sodium citrate that give equal concentrations of calcium ions, and that produce an equal reduction in the response of the frog's heart can be measured in Figs. 1 and 2. Such figures are shown in Tables VI and VII.

TABLE VI.

Millimolar concentrations of citrate which give equal concentrations of calcium ions in carbonate Ringer (pH = 7·0) with varying concentrations of calcium chloride.

M.molar conc. of calcium ions	Millimolar concentration of calcium chloride: 0·5	1·0	2·0	4·0	8·0
	Millimolar concentration of citrate				
(i) 0·18	0	1·3	2·1	5·0	12·0
(ii) 0·39	—	0	0·5	2·2	4·6

TABLE VII.

Millimolar concentrations of citrate which give equal reduction in the response of the frog's heart varying concentrations of calcium chloride.

Response of frog's heart expressed as p.c. of maximum response	Millimolar concentration of calcium chloride: 0·25	0·5	1·0	2·0	4·0	8·0	16·0
	Millimolar concentration of citrate						
10	0	0·5	2·0	3·7	7·8	18·5	45
40	—	0	0·7	2·2	5·0	12·5	32
85	—	—	0	0·8	2·1	7·4	18

The figures given in Tables VI and VII are related by the fact that in the absence of citrate 0·5 millimolar calcium chloride gives 0·18 millimolar calcium ions and reduces the frog's heart response to 40 p.c., whilst 1·0 millimolar calcium chloride gives 0·39 millimolar calcium ions and reduces the frog's heart response to 85 p.c. of the maximum. If the mixtures that reduce the response of the frog's heart to 40 p.c. of the maximum be taken as an example (middle line, Table VII), it will be seen that the ratios of the concentrations of calcium chloride and of citrate are as follows:

$$\frac{0{\cdot}5}{0};\ \frac{1{\cdot}0}{0{\cdot}7};\ \frac{2{\cdot}0}{2{\cdot}2};\ \frac{4{\cdot}0}{5{\cdot}0};\ \frac{8{\cdot}0}{12{\cdot}5};\ \frac{16}{32}.$$

These ratios are obviously not constant, but since the frog's heart requires 0·5 millimolar calcium chloride to give a response 40 p.c. of the maximum in the absence of citrate, this quantity may be subtracted from the calcium concentrations, and then the ratios become:

$$\frac{0}{0};\ \frac{0{\cdot}5}{0{\cdot}7};\ \frac{1{\cdot}5}{2{\cdot}2};\ \frac{3{\cdot}5}{5{\cdot}0};\ \frac{7{\cdot}5}{12{\cdot}5};\ \frac{15{\cdot}5}{32}.$$

These results suggest some relation between the concentrations of calcium and of citrate that produce equal results on the frog's heart, and when these figures are plotted on a logarithmic scale, as in Fig. 3, they give an approximately linear relation with the higher concentrations of citrate.

Examination of Fig. 3 shows that the relation between the calcium concentrations and citrate concentrations, that when mixed produce

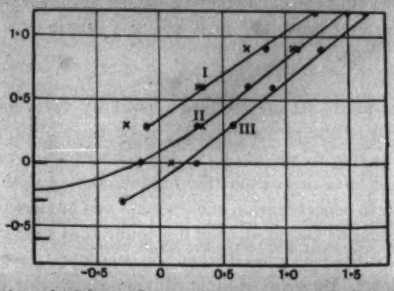

Fig. 3. Concentrations of calcium and citrate that produce equal concentrations of calcium ions, and equal responses in frog's heart. Abscissa = log of millimolar concentration of sodium citrate. Ordinate = log of millimolar concentration of calcium chloride. × = figures for equal concentrations of calcium ions. ⊙ = figures for equal reductions of frog's heart.

Curve I. Calcium ions = 0·39 millimolar; heart response = 85 p.c. of maximum.
Curve II. Calcium ions = 0·18 millimolar; heart response = 40 p.c. of maximum.
Curve III. Heart response = 10 p.c. of maximum.

either an equal reduction in the response of the frog's heart, or which contain equal concentrations of calcium ions, can be expressed as follows:

Let $[Ca_1]$ be the concentration of calcium that produces a given effect in the absence of citrate and $[Ca_2]$ the concentration of calcium that produces the same effect in the presence of citrate [Ci].

Then $K[Ci]^n = [Ca_2] - [Ca_1]$, where K and n are constants.

The value of n is about 0·9. The middle curve of Fig. 3 was drawn to this formula ($n = 0{\cdot}89$ and $K = 0{\cdot}77$).

The system investigated is unfortunately too complex for mathematical analysis. For example, the concentration of calcium ions must be affected not only by the concentration of citrate, but also by the presence of carbonate and of traces of phosphate, and there are several other factors that may have an influence. It is interesting, however, to note that the resultant of a large number of factors is a relatively simple relation between the various concentrations of calcium and the concentrations of citrate that together produce equal effects. This relation is similar to the relation that has been found for the antagonism of various pairs of drugs. (Clark(6) acetyl choline and atropine; Gaddum(7) ergotamine and adrenaline.)

The fact that this simple relationship happens to appear in the present case, where it is certain that a number of highly complex reactions are involved, shows that the existence of a simple mathematical relationship does not necessarily prove the existence of a simple type of antagonism between two drugs.

Discussion.

The effects produced on the frog's heart by variations in the total calcium content of the perfusion fluid, or by the addition of citrate can be explained as due to variations in the concentration of calcium ions, provided that it be assumed that the fluid in contact with the heart cells contains a certain concentration of carbonate and has a reaction of *p*H 7·0. If this assumption be accepted, then it is found that a maximum response is obtained from the frog's heart when the concentration of ionic calcium is 0·43 millimolar. Increase of ionic calcium above this figure produces little effect until a value of about 1·5 millimolar is attained; this value and higher values produce a marked depression of activity. Any reduction of ionic calcium below 0·43 millimolar depresses the response of the heart, and the percentage by which the response of the heart is reduced below its maximum is approximately equal to the percentage by which the ionic calcium is reduced below 0·43 millimolar. This is equally true whether the ionic calcium be reduced by reduction of the total calcium content or by the addition of sodium citrate.

The effect of variations in ionic calcium on the force of response of the heart can be explained most simply on the hypothesis that the response of the heart is dependent on the presence in the surface membranes of calcium soaps or of calcium lipoid complexes, which are highly labile and dissociate immediately the ionic concentration of calcium in the perfusion fluid is reduced. This hypothesis affords a possible explanation of the apparently divergent effects produced by variations in calcium content on blood coagulation and on the response of the frog's heart. Blood coagulation depends on a non-dialysable calcium complex, possibly a protein or lipoid complex. Removal of the calcium ions does not prevent coagulation from taking place presumably because the essential calcium complex does not dissociate sufficiently rapidly. The calcium lipoid complex in the frog's heart must, however, on the above hypothesis, be supposed to dissociate extremely rapidly when the ionic calcium is reduced, with the result that reduction in the concentration of calcium ions below a certain figure produces an immediate and roughly proportional effect on the response of the heart.

SUMMARY.

We have shown that the effect on the frog's heart of variations in calcium content, or of the addition of sodium citrate can be explained by the alterations produced in the concentration of calcium ions.

The mechanical response of the frog's heart varies directly as the concentration of calcium ions, when the latter falls below a value of about 0·43 millimolar.

On the other hand the process of blood coagulation is not inhibited by considerable reduction in the ionic concentration of calcium. This apparent contrast between the two processes (response of the frog's heart and coagulation of blood) can be explained on the assumption that both depend upon the presence of fixed calcium, but that the calcium is fixed in the blood in a stable condition, whilst on the surface of the heart cells it is present as a highly labile compound.

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THE AUTOMATIC REGULATION OF GASTRIC ACIDITY.

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Introduction.

During recent years the application of the method of fractional gastric analysis has yielded many interesting points in connection with gastric secretion, not the least among which is our knowledge of the variation in the acidity of the gastric contents during digestion. After taking a test-meal there is a rise in the hydrochloric acid concentration in the contents of the average healthy stomach for approximately one hour, followed by a fall, either immediately, or after a short time, to the more or less low value found in the resting organ. The maximum value is subject to variations in different subjects, but is in the region of 0·2 p.c. hydrochloric acid (0·5–0·6 *N*/10).

We must consider briefly the present state of our knowledge of this process. It has long been known that acids, salts and solutions of other substances undergo a change in concentration after introduction into the stomach. An inquiry into the mechanism of this change was taken up towards the end of the last century by several workers (Meade-Smith(1), von Mering(2), Roth and Strauss(3)). These workers formed the opinion that there were three factors principally concerned in the alteration of solutions in the stomach: (i) diffusion-interchange between the gastric contents and the blood, (ii) the specific secretion of the stomach (containing HCl and pepsin), and (iii) a "Verdünnungssekretion" having little or no acid properties.

At about this time interest was centered around the question of gastric acidity and many new observations were being made on the changes occurring in the gastric contents during digestion. Justesen(4), employing the fractional method of observation, studied the relationship of chlorides and hydrochloric acid during digestion. He observed the parallelism between acid and total chloride during the early stages of digestion and the divergence later when the acidity falls. Pfaundler(5), who also had observed that the curve of acidity falls at the end of digestion while the curve of neutral chloride rises, concluded that a certain quantity

of the secreted HCl was neutralised by an alkaline fluid secreted by the pyloric part of the stomach. Justesen was not inclined to accept this explanation, but suggested as an alternative an actual secretion of neutral chloride by the stomach. This view was strengthened by the work of Rosemann(6).

It must be remembered that Pavlov's(7) statement, that the acidity of the gastric juice as secreted in dogs had a constant acidity, exercised a profound influence upon the thought of these times, an influence which persisted in spite of many observations which were definitely in conflict with that conception. Although Pavlov believed that mucus was responsible for the fluctuations in acidity of juice secreted by the isolated pouch, he nevertheless admitted that the possibility of some relation existing between the rate of secretion and the degree of acidity was not wholly excluded.

It was known that the acidity of the contents of the digesting stomach was maintained at a much lower level than that of the pure juice, and as it did not appear likely that mucus alone was responsible for this, other factors were sought. A few years later Boldyrev(8) described experiments on dogs which seemed to throw light on this problem. He concluded from his observations that regurgitation of alkaline fluid from the duodenum into the stomach regulated the acidity by neutralisation. The subsequent experimental work of Spencer, Meyer, Rehfuss and Hawk(9), Apperly(10), Bolton and Goodhart(11), seemed to confirm, in the main, the conclusions of Boldyrev. Hicks and Visher(12), however, were not able to satisfy themselves that duodenal regurgitation was the sole factor concerned in the reduction of gastric acidity while, from experiments on man, Cröhn(13) agreed with the earlier workers that the secretion of a neutral gastric juice played an important part.

Recently Baird, Campbell and Hern(14) have carried out a series of interesting experiments using simultaneous intubation of the stomach and duodenum. They were able to show that the acidity of the stomach contents was regulated perfectly well even when the duodenum was continuously drained by suction; moreover, acid introduced into the stomach was reduced in strength under these conditions even when the fluid from the duodenum was acid. They concluded that neutralisation took place in the stomach by an alkaline secretion, probably from the pyloric region as suggested by Pfaundler.

From observations already published we have concluded that gastric acidity is not normally regulated by neutralisation, but that the stomach itself is capable of modifying the acidity of its contents according to the

needs of digestion (MacLean and Griffiths(15)). In the course of the present investigation we carried out some experiments in which solutions of varying composition were introduced into the stomachs of normal persons. Acetic acid, hydrochloric acid as well as $NaHSO_4$ and NaCl were employed. By the use of various devices we satisfied ourselves that the production of hydrochloric acid by the gastric glands is a self-limiting process; that at a point near the end of digestion the acid already accumulated in the stomach in some way stops the further production of acid and provokes the secretion of a neutral, chloride-rich fluid, which gradually displaces the acid from the stomach by dilution. This automatic regulation of gastric acidity by the stomach is seen in the following experiments.

Experimental.

1. *Acetic acid.*

Exp. 1. Fig. 1 is a graphical representation of typical findings after introducing acetic acid into the stomach. After removing the resting

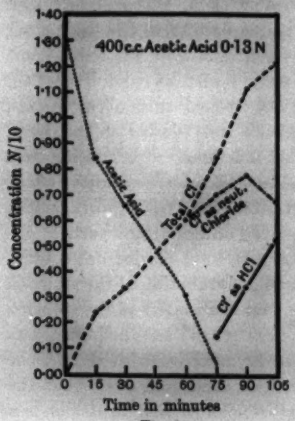

Fig. 1.

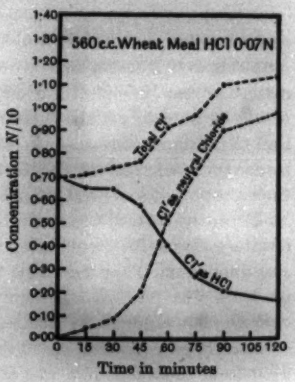

Fig. 2.

Fig. 1. Effect of acetic acid on gastric secretion.
Fig. 2. Effect of hydrochloric acid on gastric secretion.

juice from the stomach, 400 c.c. of 0·13 *N* acetic acid were introduced through the stomach tube. The total acidity of the fractions was de-

termined by titration with sodium hydroxide using phenolphthalein as indicator. Total and neutral chloride were determined, the amount of HCl being expressed by the difference between these values.

It will be seen that there was a fall in concentration of acetic acid so that it was practically absent from the stomach 75 minutes after the beginning of the experiment. Accompanying this fall in acetic acid was an increase in the chloride content of the samples; the total and neutral chloride were identical during the first 60 minutes, after which there was a divergence, signifying a secretion of HCl from that point.

This experiment illustrates the effect of acetic acid on the secretion of HCl by the stomach. The output of HCl was suppressed until a marked fall in acetic acid concentration had occurred, when secretion of HCl started and continued until difficulty was experienced in obtaining more fluid from the stomach. It is important to note that during the fall in acetic acid, although no acid appeared there was a decided increase in the neutral chloride in the gastric fluid.

2. *Hydrochloric acid.*

Having confirmed that acetic acid has an inhibitory effect on the secretion of HCl we proceeded to try hydrochloric acid.

Exp. 2. 560 c.c. of shredded wheat meal containing 0·07 *N* HCl were introduced into the empty stomach through a gastric tube. Samples were removed at 15 minute intervals until the stomach was empty. The acidity of the samples was estimated by titration and the total chloride determined. The results are shown graphically in Fig. 2. After an initial lag, a sharp fall in acidity occurred and continued until no further fluid could be obtained. The total chloride, on the other hand, climbed gradually, so that in 1½–2 hours after taking the acid it had reached the normal maximum. The course of the neutral chloride shows very clearly that as the HCl disappeared from the stomach its place was taken by neutral chloride in roughly equivalent amount.

In another experiment (Exp. 3) the acid was not introduced into the resting stomach, but secretion of gastric juice was stimulated first by 300 c.c. of 5 p.c. alcohol. A sample was removed from the stomach after 15 minutes, and after 30 minutes the stomach was emptied as completely as possible, and 200 c.c. of 0·08 *N* HCl introduced through the tube. The analyses (Fig. 3) show that although active gastric secretion was in progress, the acid put into the stomach suffered rapid reduction in strength; the fall set in immediately. The curves of total and neutral chloride exhibit similar features to those of Fig. 2. The samples were

examined for trypsin, and it was found that no tryptic activity was demonstrable during the fall in acidity. This fact, coupled with the absence of bile, serves to exclude duodenal regurgitation.

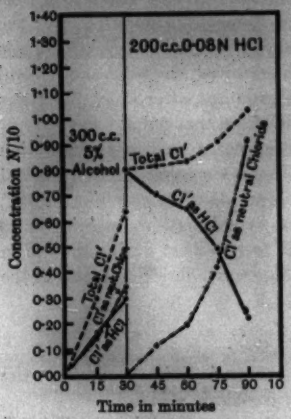

Fig. 3.

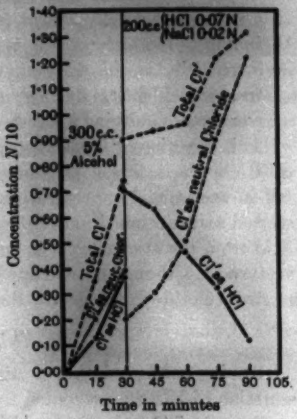

Fig. 4.

Fig. 3. Effect of hydrochloric acid administered after stimulation of secretion by alcohol. Samples removed at 45 minutes and onwards exhibited no tryptic activity.

Fig. 4. Effect of a mixture of hydrochloric acid and sodium chloride after stimulation of secretion by alcohol.

3. *Hydrochloric acid and sodium chloride.*

The effects described above show very clearly that when HCl is introduced into the normal stomach, either in the resting state or during active secretory activity, its concentration is reduced, the loss of HCl from the gastric contents being made up, step by step, by neutral chloride. Further experiments were carried out in order to study the effect of introducing sodium chloride along with the hydrochloric acid. The procedure was the same as in Exp. 3—that is to say, secretion was stimulated by 300 c.c. of 5 p.c. alcohol, and 30 minutes later, when the gastric glands were in a state of high activity, the stomach was emptied and the solution of acid and salt introduced through the tube. The following are typical experiments:

Exp. 4 (Fig. 4): 200 c.c. of solution HCl 0·07 *N*, NaCl 0·02 *N*, total chloride 0·09 *N*.

Exp. 5 (Fig. 5): 200 c.c. of solution HCl 0·068 *N*, NaCl 0·115 *N*, total chloride 0·183 *N*.

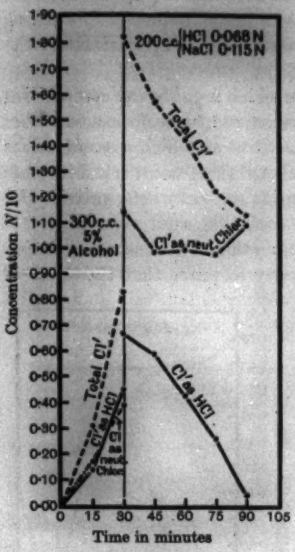

Fig. 5.

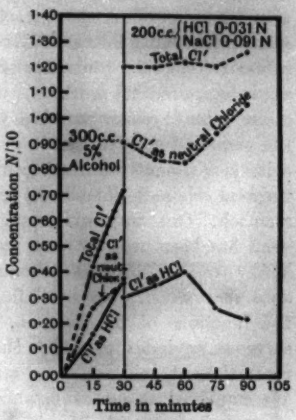

Fig. 6.

Figs. 5 and 6. Effects of mixtures of hydrochloric acid and sodium chloride after stimulation of secretion by alcohol.

Exp. 6 (Fig. 6): 200 c.c. of solution HCl 0·031 *N*, NaCl 0·091 *N*, total chloride 0·122 *N*.

The observations were similar in all respects to the previous experiments with HCl alone, except that the neutral chloride starts from a higher level because the solution contained some sodium chloride.

In Exp. 5 the fluid used was considerably above the normal maximum chloride strength of the gastric secretion. The total chloride was reduced by the stomach until in 45–60 minutes it was in the region of the maximum value. As before, the acidity fell, but it will be observed that the neutral chloride suffered little or no change during the course of the experiment.

Exp. 6. Fig. 6 shows the result of putting acid of concentration below the normal maximum attained by the subject of these experiments (0·05–0·06 *N*). In this case the acidity did not fall immediately as in the previous experiments, but continued to rise for 30 minutes before taking a downward course. The total chloride remained at approximately the same level throughout the experiment.

So far we have described the changes which occur in the composition of the gastric contents following the ingestion of hydrochloric acid, either alone or with sodium chloride. It is clear that with high concentrations of acid the acidity falls and that with this fall there is a marked increase in neutral chloride, whilst the total chloride content of the gastric juice tends to approach a level.

In order to satisfy ourselves that no secretion of acid occurred during the period of observation, it was necessary to prove that the curve of acidity as found was due entirely to the acid originally introduced into the stomach. One way which suggested itself has been used by other workers. This was to add to the acid some inert substance which, while not influencing the functions of the stomach, would serve as an index to which the concentration of acid could be referred. We employed as such substances sulphate and glucose. Neither is absorbed from the stomach when in dilute solution and they have the advantage of being easily estimated.

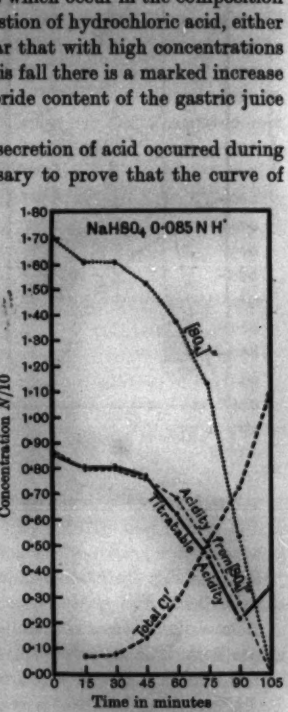

Fig. 7. Effect of sodium hydrogen sulphate on gastric secretion.

Exp. 7. Fig. 7 shows a typical experiment in which $NaHSO_4$ was used. 570 c.c. of shredded wheat test-meal containing $NaHSO_4$ were introduced into the resting normal stomach. The initial concentration of SO_4'' was 0·17 *N* and of $H^{\cdot}$ 0·085 *N*. Samples were removed from the stomach at intervals of 15 minutes and analysed for acidity (by titration), SO_4'' and Cl'. After an initial delay, the concentration of both acid and sulphate suffered a marked fall, while the chloride correspondingly rose; moreover, the acidity calculated from the concen-

tration of sulphate at each point agreed with the acidity as determined by titration within the limits of experimental error.

From this experiment it is clear that for 90 minutes no secretion of acid had occurred, otherwise the acidity of the samples would have been greater than the theoretical values derived from the sulphate; on the other hand, no neutralisation of acid could have taken place, for this would have resulted in a decrease in acidity as compared with the calculated values. Similar results were obtained in other experiments in which glucose was used as the reference substance. The stomach of a normal subject was first stimulated by 300 c.c. of 5 p.c. alcohol. After 30 minutes the stomach was emptied and 200 c.c. of 0·1 *N* HCl containing 2 g. of glucose were introduced. By withdrawing 10–20 c.c. from the stomach into a syringe and re-injecting it again, repeating this procedure two or three times, the contents of the stomach were mixed thoroughly; a few c.c. of the final aspiration were saved for analysis as a control.

It will be seen from the figure (Fig. 8) that here again precisely

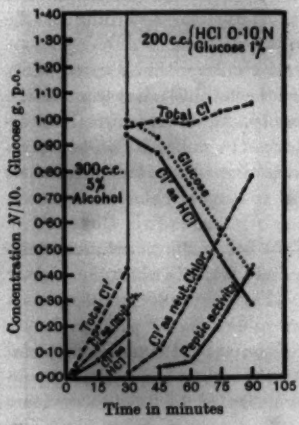

Fig. 8.

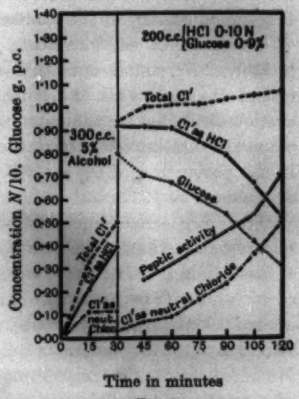

Fig. 9.

Figs. 8 and 9. Effect of hydrochloric acid, after stimulation of secretion by alcohol, showing parallel fall in concentration of glucose and rise in peptic activity.

similar results were obtained; the fall in acidity was accompanied by a parallel fall in glucose concentration. Fig. 9 is from a similar experiment on another normal individual. In the last case, because of a less rapid

emptying of the stomach, the fall in acidity was not so steep but inhibition of acid secretion was complete.

That the subjects used had healthy stomachs capable of secreting acid was confirmed by a separate fractional test-meal. In the two experiments last described, the peptic activity of the samples was estimated; there was a marked increase in this activity as the acid fell.

In none of these experiments was there regurgitation of bile into the stomach; occasionally regurgitation happened during an experiment, but because we desired the uncomplicated gastric process, such experiments were discarded. Exps. 3–6 were carried out on the same subject, who was thoroughly accustomed to the procedure; emotional factors were absent.

Discussion.

The principal constituents of the gastric juice as obtained during fractional gastric analysis or by other methods, are hydrochloric acid, neutral chloride and pepsin; large variations in the relative amounts of these substances are observed from time to time according to the functional activity of the stomach.

The total Cl′ in the resting juice varies only within narrow limits (0·11–0·12 *N*), and it is to this level that the total Cl′ rises towards the end of digestion, when the stomach is empty. Moreover, throughout digestion the value for the pure juice remains the same, as can be shown when the diluting effect of the test-meal is allowed for; large variations in the proportion of Cl′ as HCl, and Cl′ as neutral chloride, however, do occur.

Certain gastric stimulants, such as alcohol in dilute solution, temporarily raise the total Cl′ to approximately 0·13 *N*, while the highest values reaching 0·14 *N* have been observed after subcutaneous injection of histamine (Steinitz(16)). These high values should perhaps be regarded as abnormal; they are produced only by the more powerful stimulants in the normal stomach. On the other hand, Stary and Mahler(17) state that ingestion of large volumes of fluid causes secretion of a gastric juice deficient in chloride.

Under conditions of moderate stimulation, as after the ordinary wheat test-meal, total chloride values above 0·12 *N* are seldom encountered in the normal stomach though higher values are usually found in cases of hyperacidity.

After ingestion of a test-meal, the stomach strives, by its motor activity, to empty the organ and by its secretory activity to bring the

fluid contents back to the resting condition in which little or no acid is present, but a relatively large amount of neutral chloride.

Examination of the juice secreted from the Pavlov pouch in the dog (Rosemann(6), MacLean, Griffiths and Williams(18)) shows that during the first phase of digestion the hydrochloric acid rises and the neutral chloride falls; in the second phase this is reversed, the total Cl′ concentration remaining constant throughout. In the human subject the initial change is usually masked by the dilution effect of the stimulating meal, but the final change, namely, the fall in acidity and rise in neutral chloride, is commonly observed at a time when the total Cl′ is either at or within a short distance of the normal maximum.

Our earlier experimental work led us to conclude that the gastric glands secrete at all times a juice of constant Cl′ concentration, in which the relative amount of basic ions ($Na^{\cdot}$, etc.) and $H^{\cdot}$ in combination with the Cl′ were varied according to the requirements of the stomach. In other words, the gastric glands, supplied with sodium chloride by the blood, convert so much of this salt to hydrochloric acid as serves digestion (MacLean and Griffiths(15)). Heilmeyer(19) has stated that the secretion of hydrochloric acid and the secretion of chloride are two different cell functions. Steinitz(16) conceives that between two digestive phases the acid-forming power of the gastric glands is in abeyance, the chloride secretion continuing meanwhile, and that the fall in acidity at the end of digestion with the total Cl′ remaining at a high level, is an expression of the cessation of acid formation with continuance of chloride flow.

Morrell Roberts(20), in order to explain the effect of atropine on gastric secretion, supposed that in addition to a secretion of neutral chloride, there is also a special secretion of chloride which is potentially for the production of acid. He states that when there is a decrease in gastric acidity one must suppose "that a secretion of chloride already occurring along with the acid secretion was increased in concentration proportionately to the fall in concentration of acid. The latter suggestion implies a chloride secretion independent of that of acid, which may, however, appear to show varying degrees of dependence by the superimposition of the alternate secretion of chloride for acid and *vice versâ*." This is in agreement with the views of Heilmeyer(19) and Katsch and Kalk(21), and is, we believe, well in accordance with the facts.

From a consideration of these points, it will be obvious that there is a striking similarity between the chemical changes in the gastric contents after taking acid by mouth and during the final stages of digestion. We

have seen that acid introduced into the stomach from without undergoes a fall in concentration, and that this fall is accompanied by an increase in neutral chloride in such a way that the total Cl′, whether higher or lower initially than the normal average level, tends to approach this.

It is clear then, that except when the total Cl′ is initially higher than the normal maximum, the picture presented by these experiments is indistinguishable from that of the final stage of a gastric analysis.

We must now consider the matter more particularly from the standpoint of acidity. Vandorfy(22) found that acetic acid in certain concentrations determined by the functional type of stomach could completely suppress the secretion of HCl. By removing samples from the stomach at intervals after introduction of the acid, he found, as we have done, that the concentration of acetic acid fell progressively and that after an interval of time, which varied with the functional capacity of the stomach and the initial concentration of acid, hydrochloric acid appeared.

The greater the secretory power of the stomach with respect to hydrochloric acid, the higher the concentration of acetic acid necessary to inhibit secretion of hydrochloric acid. His results showed also an increase in chloride as the acetic acid disappeared.

As with acetic, so with hydrochloric acid, we find that with a normal stomach the effect is to inhibit gastric secretion of hydrochloric acid. Organs showing a high or prolonged secretion of acid (hyperacidity) respond only in a variable degree.

Further, not only is secretion of acid inhibited by acid placed in the stomach, but this acid is found to fall in concentration more or less rapidly. How is this fall brought about? There are two possible explanations: (1) neutralisation, (2) dilution by a neutral fluid.

Ever since the earliest determinations of chloride in gastric samples, any fall in acidity has, as already indicated, been generally ascribed to neutralisation by regurgitated duodenal contents, because the fall is concomitant with a rise in neutral chloride. But, in the majority of cases in which this happens, although there is a rise in neutral chloride, the total chloride concentration is unaffected or may even rise. Clearly then, there is something more than neutralisation, for in addition to alkali, neutral chloride must have been introduced into the stomach to maintain the total Cl′ concentration. This has been pointed out by Morrell Roberts.

Baird, Campbell and Hern(14) found that a decrease in gastric acidity occurred when duodenal regurgitation was excluded and, re-

jecting the possibility that the stomach could secrete neutral chloride, they were inclined to the view that an alkaline pyloric secretion was responsible.

As we have shown already, no significant neutralisation occurs in the stomach; additional evidence is furnished by the experiment with sodium hydrogen sulphate described above. After all, it would be a most irrational procedure if the stomach first elaborated acid and then, because it had produced overmuch, was forced to secrete alkali to overcome it.

Since neutral chloride appears in the stomach, whether neutralisation does or does not occur, we must consider the possible sources of this substance. It may come from the mouth (saliva), the duodenum or the stomach itself.

The saliva contains very little chloride; its entry into the stomach may be prevented by abstention from swallowing. With regard to duodenal chloride, regurgitation would certainly cause an influx of chloride into the stomach as the Cl' concentration in the duodenal fluids is approximately equal to that of the stomach (Baird, Campbell and Hern(14), Steinitz(16)), but regurgitation is only occasionally responsible.

We are therefore left with the only possibility, namely, that the stomach secretes sodium chloride, as we have shown already (MacLean and Griffiths(15)). Justesen(4) suggested the possibility of this, Rosemann(6) proved that it occurred in dogs, and more recently Heilmeyer(19), Katsch and Kalk(21), Hansman, Day and Clifton(23), Steinitz(16) and others have brought forward evidence in support of this conception. There can be no doubt that it is true.

If we suppose that in the experiments described above the gastric glands respond to the presence of a high concentration of acid in the stomach by secreting a neutral fluid containing chloride, then the interpretation of the gastric test-meal findings is clear; it is simply a question of dilution.

Anyone who has carried out on the same person a series of fractional gastric investigations must have observed the great similarity in the results. Bell and MacAdam(24) found very similar acid curves on successive days using the same subject. McClendon(25) studied the changes in $H^{\cdot}$ concentration in the stomach by means of an ingenious hydrogen electrode so constructed that it could be lowered into the stomach. He observed curves of the $H^{\cdot}$ concentration during digestion of ordinary daily meals of great variety, using this electrode and also by removing samples from the stomach by a bucket-like strainer. The results proved that the height to which the acidity rises is a personal

characteristic; the curves for different meals attained approximately the same level and their form was similar and governed by the amount of protein in the meal. Standish, Cowgill and Shohl(26) have studied the $H^{\cdot}$ concentration of the gastric contents during digestion in dogs. They found that with milk meals of different concentration the amount of hydrochloric acid secreted depends directly upon the volume and concentration of the meal, being in all cases sufficient to raise the *p*H to between 4·1 and 3·9; the *p*H of the stomach contents then tends to remain practically constant until the end of digestion.

McClendon observed that the acidity, after reaching the characteristic maximum, remained constant at least until the majority of the food had left the stomach, when the acidity usually falls because of decrease or cessation of acid secretion, and, in his opinion, entry of saliva into the stomach. Kahn and Yaure(27) similarly determined the *p*H of the juice both in the main stomach and in a Pavlov pouch in the dog and found a similar plateau of acidity. These authors suggested that secretion of acid followed an "all or none" rule.

All the evidence goes to show that the regulation of gastric acidity cannot be a result of fortuitous circumstances, such as duodenal regurgitation, but that it must be under the control of a delicate mechanism which functions with great precision. We believe our experiments justify the conclusion that secretion of hydrochloric acid by the gastric glands is a self-limiting process in that, as the gastric acidity increases it approaches a point at which, in virtue of its concentration, further secretion is stopped. It is interesting to note that Pavlov(7) observed in his animal experiments that hydrochloric acid prevented secretion of gastric juice; he says (p. 115), "With the same dogs we also discovered a new form of auto-regulation on the part of the stomach, which concerns the secretion of hydrochloric acid. It appears that the acid prevents the further secretion of gastric juice when it has accumulated in any considerable quantity in the cavity of the organ." Apperly(10) also observed that a foreign acid could suppress gastric secretion of acid.

From our experimental work we must suppose that during digestion the rising acidity of the gastric contents is arrested at a certain level by interruption of the secretion of hydrochloric acid, and that this level is maintained for a variable time until secretion of a neutral fluid sets in and reduces the gastric acidity to the fasting level. This neutral fluid probably corresponds to the "Verdünnungssekretion" of Strauss (Steinitz(16)).

Systematic investigations of the relation of peptic activity to the other constituents of the gastric juice have been few and the literature on the subject is mostly foreign. In the later experiments in this series we made some observations on peptic activity (Figs. 8 and 9). We found that accompanying the rise in neutral chloride there was an increase in peptic activity running more or less parallel with the neutral chloride. This relationship was so striking that we were induced to extend our investigations to ordinary fractional gastric analysis. In conjunction with Major T. A. Hughes, I.M.S., we made an extensive study of the problem, both in normal and pathological stomachs. Our findings, together with the methods employed, will be published in due course; we will only indicate briefly the general conclusions.

We found that the curves of peptic activity and hydrochloric acid showed no relationship during digestion; there was, however, a close association between the output of neutral chloride and peptic activity. Of particular interest in the present connection is the finding that when the gastric acidity falls near the end of digestion, the corresponding rise in neutral chloride is associated with a rise in peptic activity, exactly as shown in the graphs reproduced in this paper. This is an additional point of similarity between the terminal stages of digestion as shown by the test-meal, and the changes which take place when acid is introduced into the stomach *per os*.

We have shown that acetic acid, hydrochloric acid and sulphuric acid are capable of inhibiting gastric secretion of hydrochloric acid under certain conditions. These acids differ in their effect in one respect, namely, that while hydrochloric acid and sulphuric acid have a permanent influence, acetic acid appears to inhibit secretion only for so long as its concentration remains at a high level; when low levels are reached secretion of hydrochloric acid re-commences.

Conclusions.

1. The concentration of hydrochloric acid in the normal stomach is regulated automatically by the $H^{\cdot}$ concentration of the gastric juice. The presence of a certain concentration of acid in the stomach inhibits secretion of acid, with the result that a neutral fluid containing chloride is secreted, which, by dilution, reduces the acidity of the gastric contents.

2. Inhibition of acid secretion is produced when hydrochloric, sulphuric and acetic acids are introduced into the stomach in concentration greater than the maximum acidity normally found in the stomach.

3. Whenever a fluid is taken into the stomach, this organ, by the

combined efforts of emptying and secretion, tends to adjust the concentration of Cl′ to the normal maximum level (0·11–0·12 *N*).

4. The peptic activity of the stomach fluid rises parallel with the increase in neutral chloride which occurs along with diminishing acidity.

5. The relation of these findings to normal gastric digestion is discussed.

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A VASO-DILATOR ACTION OF ACETATES.

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The action here described was discovered accidentally. We had under investigation a depressor substance occurring in extracts from blood, obtained under certain experimental conditions. The original alcoholic extract, after concentration, was treated with basic lead acetate, to effect a further purification. Excess of lead having been removed by H_2S from the filtrate, the latter was neutralised with NaOH. When the action of this neutral extract was tested on the blood-pressure of a cat, it was found that its depressor effect was unusual in form: instead of the rapid recovery of blood-pressure following the initial fall—such as occurs after injection of small doses of histamine or choline—there occurred, after a sharp initial fall, a slow recovery.

Further experiments were made in which known amounts of histamine were added to normal blood, and the blood then extracted, in one of two ways:

(*a*) by the use of Schenk's reagent only;

(*b*) by Schenk's reagent, followed by a further purification with basic lead acetate.

In extracts (*a*) histamine was found to have been recovered almost quantitatively. Extracts (*b*), on the other hand, had acquired a more powerful and more prolonged depressor action than was produced by pure histamine, in the doses which had been originally added to the blood.

There were two possibilities: the treatment with lead acetate might have caused the liberation of a depressor substance from a previously inactive constituent of the extract, or it might have introduced a new depressor substance from without. The latter possibility was easily tested. The only substance newly added by the treatment was acetic acid, liberated in removing the excess of lead as sulphide, and converted into sodium acetate by the neutralisation; and it was found, in fact, that the addition of sodium acetate, in quantities roughly equivalent to those inevitably produced in the lead treatment, to dilute solutions of

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pure histamine, produced an increase and prolongation of depressor effect precisely similar to that under discussion. Thus, a combined injection of 0·0025 mg. histamine (·0075 mg. histamine acid phosphate) plus 27 mg. neutral sodium acetate, produced a depressor effect nearly equal to that of 0·005 mg. histamine (Fig. 1, *A*).

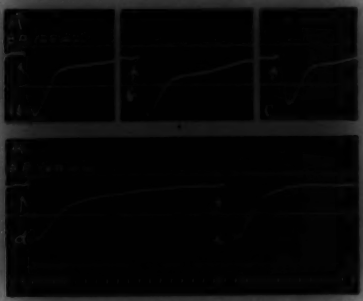

Fig. 1. Cat's blood-pressure. *A*. At (*a*), intravenous injection of 0·005 mg. histamine; (*b*), 0·0025 mg. histamine and 27 mg. sodium acetate; (*c*), 0·0025 mg. histamine. *B*. At (*d*), intravenous injection of 0·005 mg. histamine; (*e*), 0·00125 mg. histamine and 27 mg. sodium acetate and 17 mg. KCl. Time marker in all tracings shows intervals of 10″.

The observations above described had more than an immediate significance. In the course of investigations, for some time in progress in this laboratory, on the depressor constituents of certain tissue extracts, it has been the usual practice to assay these extracts quantitatively, by a physiological test on the cat's blood-pressure, during different stages in their purification. A knowledge of the depressor effects of acetates might throw light on anomalies which have appeared, from time to time, after chemical procedures involving their introduction.

General character of the depressor action. In its usual form, the depressor effect of a small intravenous injection, such as 0·25 c.c. to 0·5 c.c. of a normal solution of neutral sodium acetate into a cat, is characteristic, and consists of an initial sharp fall of 3–5 mm., followed by a momentary check, and by a more gradual fall and a still slower recovery (Fig. 2). Sodium acetate in these doses does not always produce the initial slight and rapid fall; this, on the other hand, often occurs after the injection of other hypertonic salt solutions in similar amounts, and is apparently a non-specific effect. The secondary gradual fall and recovery has not

been observed with any salts that have been tested, other than acetates. The extent of the fall of blood-pressure after a given dose of sodium acetate varies from one cat to another, and in the same cat at different stages in the experiment; in this respect its action resembles that of more potent depressors, such as histamine and choline.

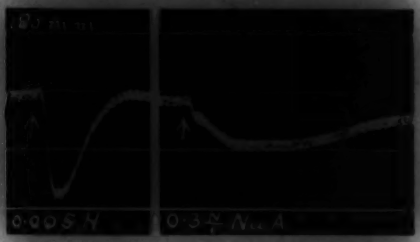

Fig. 2. Cat's blood-pressure. Intravenous injection of 0·005 mg. histamine, compared with that of 0·3 c.c. of *N*/1 neutral sodium acetate.

Mechanism and location of the depressor action. The following observations contributed to the analysis of the effect.

1. A pronounced and definite depressor effect, such as that obtained by injecting 0·5 c.c. of normal sodium acetate into a cat, was not accompanied by any perceptible change in the rate of the pulse. Accordingly it seemed probable that the effect was mainly, if not entirely, due to a dilator action on the peripheral vessels.

2. This was confirmed by direct experiments.

(*a*) In a cat under ether, with arterial blood-pressure recorded from the carotid artery, the volume of one hind limb, freshly denervated by section of the sciatic and crural nerves, was registered by a plethysmograph and bellows-recorder. Heparin was given and a cannula for intra-arterial injection introduced, through the iliac artery of the other side, into the aorta just central to its bifurcation, as described by Dale and A. N. Richards(1). A small injection of histamine into the aorta by this route produced the usual expansion of the limb, followed by a relatively small fall of the general arterial pressure. Sodium acetate solution produced similar evidence of vasodilator action when injected in the same way, the effects, as with ordinary intravenous injection, being more prolonged in proportion to their intensity than those of histamine. The action of sodium acetate was accordingly, like that of histamine, a peripheral effect on the vessels, independent of the nervous system

(Fig. 3). It will be seen that the expansion of the limb in response to the acetate is preceded by a sharp, evanescent constriction. This is a non-

Fig. 3. Cat's blood-pressure (below), and plethysmographic record of limb volume (above). Intra-arterial injection of 0·0025 mg. histamine, compared with those of 0·5 c.c. *N*/1 sodium acetate, and of 0·5 c.c. *N*/1 potassium acetate.

specific effect of the hypertonic solution, and is seen with similar injections of normal solutions of salts other than acetates.

(*b*) A dog's isolated hind limb was perfused with the Dale-Schuster pump(2), the dog's own blood being used, and the venous blood oxygenated by perfusion through the lungs with the second pump of the system. An attempt to obtain significant plethysmograph records failed, any fall of the arterial pressure, however produced, being accompanied by passive shrinkage of the part of the limb in the plethysmograph. The record of changes in the pressure in the arterial cannula showed, however, quite adequately the changes in peripheral resistance, which alone could affect the pressure produced by the uniformly acting pump. Fig. 4 shows the

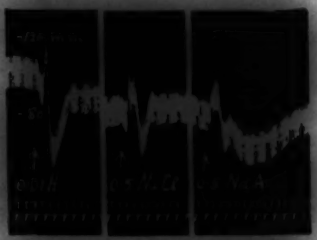

Fig. 4. Artificial perfusion of isolated dog's hind limb. Record of arterial pressure (above), rate of outflow, and time. Comparative effects of intra-arterial injections of 0·01 mg. histamine, 0·5 c.c. of *N*/1 NaCl, and 0·5 c.c. of *N*/1 Na-acetate. Outflow recorder in this and Figs. 5 and 6 marks volumes of 10 c.c.

effect of injecting, into the blood going to the arterial cannula, 0·5 c.c. of normal solutions of NaCl and NaĀ respectively. The former produces, like all hypertonic salt solutions which we have tried, a sharp, evanescent fall of pressure. With sodium acetate solution this effect of mere tonicity is followed by a specific, and quite definite, slower fall and recovery, indicating a vaso-dilator effect similar to that seen on the peripheral vessels of the whole cat.

3. The effect of sodium acetate on the blood-pressure was unaffected by the previous administration of atropine, in doses sufficient to abolish the action of choline or its esters in ordinary doses. The activity of acetates on the blood-pressure could, accordingly, not be related to the peculiar potency of acetyl-choline, as suggested by Le Heux(3) in explanation of the stimulating effect of acetates on intestinal activity. We shall see that the depressor effect of acetates resembles that of histamine rather than that of choline.

4. The depressor action being due to peripheral vaso-dilatation, it was desirable to identify more closely the size of the vessels participating in the response. It was stated by Dale and A. N. Richards(1) that a preparation of the isolated mesenteric arterial branches of the cat, perfused with blood, shows a good vaso-dilator response to acetyl-choline, but only constriction with histamine. According to Burn and Dale(4) the similar arterial preparation from the dog shows some vaso-dilator response to histamine as well as to acetyl-choline. The effect of acetate has been tried on both.

(*a*) The preparation of the cat's mesenteric arterial branches, perfused with whipped cat's blood by means of the Dale-Schuster pump, gave a full vaso-dilator response to acetyl-choline, and a just perceptible trace of vaso-dilatation with very small doses of histamine, larger doses of the latter substance producing vaso-constriction. With sodium acetate, in any dose, it exhibited only a small vaso-constrictor effect.

(*b*) In a similar preparation of the dog's mesentery the finding of Burn and Dale was confirmed, both histamine and acetyl-choline producing vaso-dilatation. Sodium acetate in this case also gave only a small vaso-constrictor response (Fig. 5).

(*c*) An isolated strip was prepared by spiral section of iliac artery of a dog, and suspended in 100 c.c. of warm oxygenated Ringer's solution according to the familiar technique. Contraction to maximal tone was caused by addition of 0·02 gm. of adrenaline, a smaller contraction by 1 mg. of histamine, and relaxation by sodium nitrite. Sodium acetate, added in amounts up to 1 c.c. of normal solution, had no perceptible

effect in either direction. According to the indications of these experiments, therefore, the vaso-dilator effect of the acetates corresponds, in

Fig. 5. Perfusion of isolated dog's mesentery. Record of arterial pressure, volume of outflow, and time. Injections of 0·2 c.c. *N*/1 Na-acetate, 0·01 mg. histamine, and 0·002 mg. acetyl-choline.

its distribution, much more nearly to that of histamine than to that of choline and its esters; where the action differs from that of histamine in location, it appears to be even more exclusively on the minute peripheral vessels.

5. The correspondence of the action to that of histamine in its localisation could be tested in another way. In the rabbit anæsthetised with ether or urethane, in contrast to the cat under similar conditions, it is unusual to observe any depressor vaso-dilator effect with histamine. In one experiment on a rabbit under urethane we observed a full depressor effect with acetyl-choline (0·001 mg.), a very small but definite depression even with small doses of histamine (0·0025–0·005 mg.), but no trace of depressor action with sodium acetate in any dose, a small rise of arterial pressure being the only effect perceptible. Again the indication is in favour of the depressor effect of acetates being even more selectively peripheral than that of histamine.

6. Another rather striking correspondence between acetates and histamine may be mentioned, in an action not vaso-dilator. We have been engaged with Dr Dale in experiments on the response, to various conditions, of the vessels of a dog's liver, artificially perfused through hepatic artery and portal vein simultaneously. In the later stages of such experiments a small dose of histamine produces only two pronounced effects in this preparation—a rise of pressure in the portal vein, and a restriction of the outflow through the hepatic veins and vena cava. For reasons which will be fully discussed in a later publication, we have been

led to attribute these effects to that action of histamine which Mautner and Pick(5), and more recently Baer and Rössler(6), have detected, namely a stimulation to constriction of the branches of the efferent, hepatic veins. Under such conditions sodium acetate likewise causes rise of portal pressure and restriction of hepatic outflow—both due, as we believe, to constriction of hepatic venules—while salts of other acids, such as sodium chloride, in equal doses of equimolecular solutions, have no trace of this action (Fig. 6).

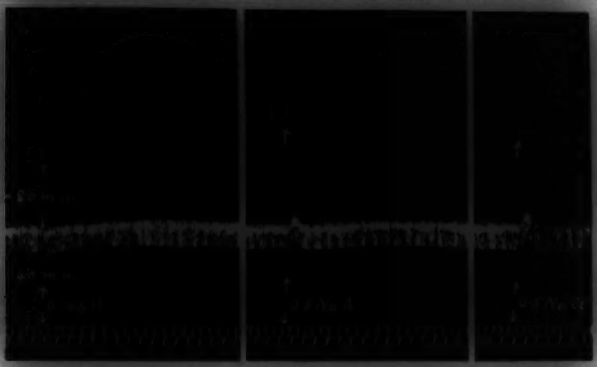

Fig. 6. Perfusion of isolated dog's liver. Records of portal vein pressure, hepatic artery pressure, and volume of hepatic outflow. Effects of intra-arterial injections of 0·005 mg. histamine, 0·5 c.c. of *N*/1 Na-acetate, and 0·5 c.c. of *N*/1 NaCl.

Effects of other acetates. The effects of the Ca^{++} and Mg^{++} ion were not sufficient, in the intravenous doses in which calcium and magnesium acetates were tested, to interfere with or modify those due to the $\bar{A}'$ ion; their depressor effects were not to be distinguished from that of sodium acetate in equimolecular dosage. Slight deviations from neutrality in either direction did not alter the action. The effects, that is to say, were due to the acetate ion, and not to the hydrogen or hydroxyl ion. Only in the case of potassium acetate, of the salts tested, did the kation contribute independently to the effect. When potassium acetate was injected intravenously, the first visible effect was the rapid fall of the arterial pressure, due to the depressor effect of potassium ions on the heart-muscle. This persisted during the vaso-dilator effect of the acetate ions, and the resulting total depression was deeper than that produced

by the equivalent of sodium acetate. When potassium acetate was injected directly into the aorta, the plethysmographic record for the limb showed an immediate vaso-constriction, which was obviously the vaso-constrictor effect of potassium salts described by Mathison(7), followed by the vaso-dilator effect of the acetate ion (Fig. 3).

From the point of view with which we began these observations it should be noted that a tissue extract, purified by the lead acetate treatment, is likely to contain excess of both potassium and acetate ions, and that the combined effect of these may produce material errors, if the histamine content is estimated from the depressor action on the atropinised cat. As Fig. 1 *B* shows, the injection of 27 mg. of sodium acetate and 17 mg. of potassium chloride with 0·00125 mg. of histamine, may produce a depressor effect not easily distinguishable from that of a dose of pure histamine four times as great (0·005 mg.).

Effect of other neutral salts. A number of other acids were tested in the form of their sodium salts, in doses of from 0·25 to 0·5 c.c. of normal solutions. None of them showed any significant effects on the cat's blood-pressure. Those tested were NaCl, $NaNO_3$, Na_2SO_4, NaI, and the lactate, butyrate, benzoate and salicylate. Only the aceto-acetate showed a depressor effect, of approximately equal depth to that caused by the equivalent of sodium acetate, but showing a more rapid recovery and a slight pressor after-effect. The same effect was obtained, whether the sodium salt or the ethyl ester was used. We are indebted to Mr W. W. Starling for the preparations of these substances.

SUMMARY.

Acetate solutions, in doses of the order of 25 mg. or more, exert a vaso-dilator effect in the dog and cat; the location of the action appears to be upon the smaller blood vessels, and is similar in this respect to the dilatation produced by histamine.

The dilator action of acetates takes place independently of the central nervous system, and is not affected by atropine.

We wish especially to express our gratitude to Dr Dale for guidance both in planning and in carrying out these experiments.

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ON THE SUPPOSED RELATION BETWEEN SURFACE AREA AND HÆMOGLOBIN CONTENT IN THE RED CELLS OF MAMMALIA.

By ERIC PONDER.

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BÜRKER has claimed that the relation

$$\frac{\text{Hb. in one red cell}}{\text{Surface area of cell}} = \text{constant}$$

is true for the red cells of certain of the mammalia (1). This finding he construes as evidence that the hæmoglobin is distributed as a surface layer in the cell. In 1923 I pointed out that this relation could not be established on the evidence existing at that time, owing to the unsatisfactory nature of the measurements of cell diameter and area (2). Recently EMMONS has re-opened the question by claiming that accurate measurements demonstrate the relation to exist, and by again concluding that the relation provides evidence of the surface distribution of the hæmoglobin (3). Both BÜRKER, who gives figures for nine animals, and EMMONS, who gives figures for four, imply that this supposed relation between hæmoglobin content and surface area is a general one for the red cells of mammalia.

Before proceeding further, it may be mentioned that there is no *a priori* reason for a linear relation existing either between hæmoglobin content and red cell area or between hæmoglobin content and cell volume. EMMONS remarks that one would expect the hæmoglobin reading to vary with the cell volume if the pigment is uniformly distributed throughout the cell, but to vary with the cell area if laid down as a surface layer, and considers the absence of a perfect linear relation between hæmoglobin content and cell volume to be a powerful reason for seeking one between the hæmoglobin content and the cell area. This expectation is not justified, unless it is assumed that hæmoglobin can exist in one concentration only. Further, a linear relation between hæmoglobin content and surface area, even if shown to exist, would not provide evidence in favour of a surface distribution of the pigment,

for such a relation might appear for many reasons quite unassociated with the mere position of hæmoglobin within the cell.

1. *The measurement of red cell area.*

The surface area of the red cell cannot be measured directly, but requires to be computed from measurements of other dimensions. Various methods of making these measurements, and various formulæ for the calculation of the cell area from them, have been proposed, but, with one exception, it has never been claimed that the resulting estimates of area are anything but approximate.

The exception referred to is constituted by the computations of Emmons, upon which the supposed relation between red cell area and hæmoglobin content rests. Emmons refers to his method as "a new method depending on Guldin's theorems," but it is in fact the same method as suggested, used, and finally abandoned by me some years ago. The method consists in outlining the cells in cross-sectional view at a known magnification, and then in computing the surface area in the usual way from the values of the length of arc and of its centre of gravity. The accuracy with which this procedure can be carried out depends, of course, on the accuracy with which the cell can be outlined; this accuracy is not very great, for the following reasons.

(i) A little consideration will show that it is an impossibility to obtain a satisfactory cross-sectional view of a body of the shape of the erythrocyte, in whatever position it may be held. Even if it is seen directly edge on, diffraction bands arising from the edges obscure the view of the biconcavities, and, what is more important, diffraction bands arising from the biconcavities interfere with the resolution of the edges. Quite apart from this unavoidable interference of the image of one part of the cell with that of other parts, it is a very difficult matter to obtain cross-sectional views of the cells at all. The only method in common use is to observe cells in rouleaux, and here again it is a matter of common experience that diffraction bands from one cell interfere with a clear view of its neighbours. In addition to this, the cells of rouleaux overlap and distort one another to such an extent that it is impossible to outline any one with certainty. This objection does not, of course, apply with the same force to the cells situated at the ends of rouleaux, and it is from these and from these only that we can construct the outline from which the cell area is to be computed, unless the cells can be obtained edge-on in a photographic plate.

(ii) Even if the red cell could be seen in cross-sectional view, the

accuracy with which it could be outlined would be limited by the fact that its edges cannot be resolved to less than about 0·2μ. This fact in itself renders estimates of area unreliable, for even when the best optical conditions are employed the error may amount to 5 p.c. or more. In the case of Emmons' computations this figure is almost certainly exceeded, for the optical system used must have been incapable of giving a high degree of resolution owing to the excessive thickness of the slide employed[1].

Although these two points of criticism are of the first importance, they do not cover the most weighty objection to the computations of area with which we are concerned, for the method of outlining the cell to scale consists in fitting the outline, at a magnification of 10,000, into a rectangle whose length and breadth represent the maximum cell diameter and maximum cell thickness respectively. The legitimacy of this procedure depends entirely, of course, on the accuracy with which the maximum diameter and the maximum thickness are themselves determined.

Emmons gives 7·8μ for the mean diameter of the human erythrocyte, and 7·2μ, 6·6μ, and 5·6μ for the diameters of the cells of the dog, rabbit and cat. All these measurements are made with the "eriometer," and on cells in dried films(4); their value accordingly depends on the accuracy of this particular method of measurement and on the absence of shrinkage of the cells during drying. Both these points have already been sufficiently discussed(5), and there is no doubt that the above figures are considerable underestimates, for the values which are given either by photographic methods or by the diffraction method recently described, are 8·8μ(6), 7·6μ, 7·3μ(7), and 6·3μ.

The values given for the maximum cell thickness are also open to dispute, for it is claimed that measurements of the cells in rouleaux show that the thickness in the four species dealt with (man, dog, rabbit and cat) is very nearly the same, and equal to 2·0μ approximately. It is further implied that this constancy exists in the case of the cells of the mammalia in general. The suggestion is not borne out by measurements of the thickness of cells of mammals other than those mentioned by Emmons, as the following table shows. The mean diameter of the cells in the fresh and in the dried state is given, together with the

[1] The slide employed is a hæmocytometer slide, the thickness of which makes it impossible to focus the illuminant on the object by means of a condenser of high N.A. The effective N.A. of the optical system is thus reduced, and the resolving power reduced in proportion.

mean thickness, and it will be seen that the latter dimension is far from constant[1]. Attention is also drawn to the inconstant shrinkage on drying, which makes the prediction of the true diameter from the dried diameter unreliable, and to the fact that the values for the diameter of the dried cells agree fairly closely with those given by Gulliver.

Animal	Diameter undried	Diameter dried	Thickness
Elephant	9·6	8·9	2·8
Spider monkey	9·2	7·5	3·1
Opossum	9·2	7·4	1·6
Man	8·8	7·8	2·7
Wallaby	8·6	8·0	2·1
Armadillo	8·5	7·9	2·2
Sapajou monkey	7·8	6·8	2·6
Skunk	7·7	6·7	2·1
Coati mundi	7·6	6·8	2·6
Rabbit	7·3	6·6	1·6
Elk	6·2	5·8	1·6
Tahr	3·3	3·0	1·3

The method used in making these measurements, which are given in μ to the first place only, requires a brief description, for hitherto satisfactory measurements of red cell thickness by photographic methods have been unobtainable. Samples of cells and of plasma are prepared from the blood of the animal, and preparations of red cells in plasma made in the manner already described (6); a much larger proportion of cells, however, is used in making the cell-plasma mixture, so that the cells of the preparation are almost in contact. In such preparations a few cells can always be seen lying on edge, and, provided that the film is thin and the vaseline seal complete, these cells will remain on edge for quite a long time. A cell selected for photography should satisfy two criteria: it should be undistorted by other cells, and it should present a perfectly symmetrical outline, the two biconcavities being equal in extent and clearly seen. If the depth of focus of the objective is about $0{\cdot}5\mu$, such a cell can be sharply focussed in cross-sectional view and photographed in the usual way. Relatively accurate measurements of the thickness, etc., can then be made from the plate, although the resolution and clearness of outline are never so good as those obtained when the cell is photographed as a disc. Although the method is a tedious one, it avoids the measurement of cells in rouleaux, which can be objected to both on the grounds of distortion of the cells and on the grounds that the cells of rouleaux are not always packed to the same

[1] It may be mentioned that the micrometer and scales used for the determination of these dimensions in absolute units have been calibrated both at the National Physical Laboratory and at the Bureau of Standards, Washington, D.C. The two calibrations agree exactly.

extent, either in different rouleaux or in different animals. The tacit assumption that distortion is absent and that packing is uniform may be partly responsible for Emmons' results.

The figures of the above table are sufficiently numerous to show that the mammalian red cells cannot be regarded, even for approximate purposes, as cylinders of constant height. It requires to be observed, however, that Emmons' treatment of the cells as cylinders of constant height is not based on measurements of area alone, but also upon measurements of cell volume, it being claimed that the calculated volume of a cylinder of $1{\cdot}8\mu$ in height and of the diameter of the cell is very nearly the same as the cell volume as found experimentally. The correspondence between the results as calculated on this assumption and the results obtained in experiment is shown in Fig. 1 of Emmons' communication, and at first sight is very striking indeed. Its significance, however, becomes somewhat less when it is observed that the volumes are those of the cells in plasma, while the diameters from which the volumes are calculated are those of the cells when dried. The correctness of the result thus once more depends on the absence of shrinkage on drying, and the agreement between experimental and calculated results shows that the method of calculation contains an error sufficiently great to compensate for the initial errors of measurement.

Even if we allow that we are able to measure the diameter and thickness of the red cell with a considerable degree of accuracy, we still require to determine which of several methods is the best for the finding of the surface area. There are at least four expressions to be considered. In the first the theorem of Pappus(8) is employed, and we have

$$\text{Area} = 4\pi l\bar{x} \qquad \ldots\ldots(1),$$

where l is the length of a quadrant of arc and $\bar{x}$ the coordinate of its centre of gravity. In the second, the cell is imagined to be circumscribed by a spheroid, and the area is given by the expression

$$2\pi A^2 + 2\pi AB\,\frac{\sinh^{-1} e}{e} \qquad \ldots\ldots(2),$$

where A is equal to half the cell diameter, B to 0·067 times the thickness, and e has the value $\sqrt{A^2 - B^2}/B$. If we put $e = 0{\cdot}\dot{6}$ as an approximation[1],

[1] Hitherto I have been content with this approximation for many purposes, partly because of the difficulty in obtaining satisfactory measurements of thickness, and partly because the necessity for measuring the cell area with great accuracy has not previously arisen. As the figures of this paper show, the approximation is anything but a good one; in fact, the correlation between the cell diameter and the cell thickness is not more than about 0·7 for the animals considered. The idea that the mammalian erythrocyte has an

this expression reduces to

$$\text{Area} = \tfrac{1}{12} A^2 \qquad \ldots\ldots(3).$$

The third method is to regard the cell as a cylinder, in which case

$$\text{Area} = 2\pi r\,(r + h) \qquad \ldots\ldots(4),$$

where r is half the diameter of the cell and h the thickness. The best way of testing the applicability of these expressions is to compare the values for cell area given by each when applied to the cells mentioned in the preceding table. The comparison is given below, the area being expressed in μ^2.

Animal	Expr. 1	Expr. 2	Expr. 3	Expr. 4
Elephant	190	187	170	229
Spider monkey	182	176	157	222
Opossum	156	148	155	177
Man	163	149	143	195
Wallaby	153	140	137	173
Armadillo	145	138	135	171
Sapajou monkey	133	126	112	159
Skunk	123	115	110	144
Coati mundi	132	122	109	154
Rabbit	110	106	99	134
Elk	79	75	71	82
Tahr	26	23	20	30

Of the four expressions considered, (1) is probably the best, for it contains no simplifying assumptions. Expression (2), however, yields results very similar to those given by (1), the greatest difference being 9 p.c. The simplified expression (3) is not so good, for the results are uniformly too low, nor is expression (4) satisfactory, for the results are uniformly too high. The significance of the difference between the values for area given by the last three formulæ and those given by expression (1) depends entirely, of course, on the accuracy with which (1) itself can be applied in actual practice, *i.e.* on the accuracy with which the red cell can be outlined in cross-section. Emmons considers the error involved in the outlining of the cell and in the application of Pappus' theorem to be less than 4 p.c. My own experience is that an error from 10 to 15 p.c. may easily arise in the course of the most careful work. It was for this reason, indeed, that the method was abandoned some years ago.

There appears to be no reason for discontinuing the use of expression (2) as a means of calculating the red cell area.

invariable shape accordingly appears to be wrong. The inconstancy of the A/B ratio makes it possible that, by the selection of certain animals, the thickness of the cells should be found to be constant; at the same time, however, it is to be remembered that the terms containing values for cell thickness contribute very little to the total area.

2. *The relation of cell area to hæmoglobin content.*

The relation of red cell area per cubic millimetre of blood to the hæmoglobin content is shown, for the animals referred to in the preceding tables, by the following figures. The hæmoglobin was measured by Palmer's colorimetric method as carboxyhæmoglobin; the red cell counts were made in the usual way. The figures are averages of from three to eight determinations.

Animal	Area, cm.²	Hb. p.c.	Hb/Area
Elephant	6·65	71	10·7
Spider monkey	10·37	76	7·3
Opossum	9·20	83	9·0
Man	8·15	100	12·2
Wallaby	12·54	91	7·3
Armadillo	6·38	90	14·3
Sapajou monkey	6·25	78	12·5
Skunk	12·08	96	7·9
Coati mundi	6·60	46	7·0
Rabbit	6·05	82	13·5
Elk	6·08	97	15·9
Tahr	2·86	44	15·6

As will be seen from the figures, the hæmoglobin/area ratio is far from constant. Expressing the relation by means of a correlation coefficient, we find the very low value of 0·3, which might easily arise by chance. In Fig. 1 the values for the total surface area are shown

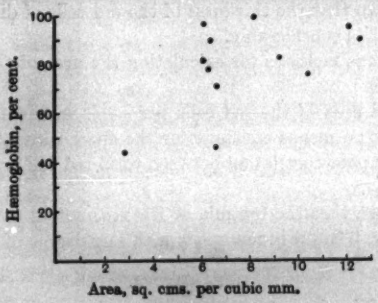

Fig. 1.

plotted against the values for hæmoglobin content; not even by making the most extravagant allowance for experimental error could the points be put on a straight line passing through the origin. These results accordingly do not support the contention of Bürker and of Emmons.

3. *The relation between cell diameter and red cell count.*

Emmons has suggested that the number of cells per cubic millimetre of blood can be calculated from the red cell diameter by means of the formula

$$N = \frac{4{\cdot}56967.(10^6)}{D(D+3{\cdot}6)} \qquad \ldots\ldots(5).$$

The following table contains experimental values for the red cell counts of the animals dealt with in this paper. These values may be compared with those calculated from expression (5).

Animal	N obs. $\times 10^{-6}$	N calc. $\times 10^{-6}$
Elephant	3·5	4·1
Spider monkey	5·7	5·5
Opossum	5·9	5·6
Man	5·0	5·1
Wallaby	8·2	4·9
Armadillo	4·4	5·0
Sapajou monkey	4·7	6·5
Skunk	9·8	6·6
Coati mundi	5·0	6·5
Rabbit	5·5	6·8
Elk	7·7	8·4
Tahr	11·0	23·1

It will be clear from this table that the red cell count cannot be calculated with any degree of accuracy by the use of Emmons' formula.

Summary.

1. It is shown that the thickness of the red cells of different species of mammals varies considerably.

2. The various methods for calculating the area of the red cell are compared.

3. The total area of the red cells per cubic millimetre of blood is shown to be by no means constant for the species investigated. There is a remarkably poor correlation between total red cell area and hæmoglobin content.

4. It is shown that the formula of Emmons does not enable us to predict the red cell count in mammals with any degree of accuracy.

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STUDIES OF REFLEX ACTIVITY IN THE INVOLUNTARY NERVOUS SYSTEM.

I. Depressor Reflexes.

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The vaso-motor effects of stimulating the central end of the vagus (cat) or the depressor nerve (rabbit) have been re-investigated in an attempt to elucidate some of the processes which take place in the nerve centres of the involuntary nervous system; many of the experiments were carried out in the light of Sherrington's "humoral" theory of reflex action(1).

Methods.

The animals employed were the cat and rabbit, anæsthetised with chloralose or ether, or decerebrated (under deep C.E. anæsthesia) through a trephine opening at various levels in the midbrain. In the case of the decerebrate animals about 1 hour was allowed to elapse after the operation to permit the initial anæsthetic to be blown off completely (cf. Vincent and Curtis(2)). Both vagi were divided in all cases to exclude reflex effects on the heart. It is advisable to do this previous to decerebration while the animal is deeply anæsthetised; section of both vagi subsequent to decerebration often gives rise to grave disturbances of respiration and of blood-pressure and may prove fatal. In my experience a separate depressor nerve in the cat is uncommon. Both vagi may be depressor (perhaps the left more so than the right) or they may give rise to a mixture of pressor and depressor effects(3); occasionally they may produce pressor effects only. The proportion of depressor to pressor fibres in the vagus of the cat seems to differ in different animals.

For stimulation I have used, in addition to the ordinary coil, a special coil kindly made for me by Prof. Russ and Mr Watters. This enables the current in the primary circuit to be varied from 5 to about 400 milliamperes, and the frequency of interruption to be varied from 2 to 50 double shocks per second. Make and break shocks were equalised by the Helmholtz arrangement. The position of the secondary coil in relation to the primary was kept fixed and the strength of shock was raised by adjusting the current in the primary.

Irregular results tend to be obtained after the nerve has been stimulated a considerable number of times, if a slight over-dose of chloralose has been given, or if Traube waves have been set up. If strong afferent stimulation has been employed to produce marked lowering of the blood-pressure these irregular responses set in earlier in the course of the experiment. Little attention has consequently been paid to the results obtained under such conditions.

The respiratory disturbances which may be set up by the vagus nerve in the cat tend to produce secondary blood-pressure changes. A considerable number of experiments were therefore carried out under artificial respiration.

Mechanical and thermal stimulation. It has been pointed out by Sharpey-Schafer(4) that the depressor nerve in chloralose anæsthesia is very sensitive to mechanical stimulation (cf. Fig. 10); in my experience the same is frequently true of the depressor nerve in the decerebrate animal (Fig. 1). Care was therefore taken to avoid traction and the nerve

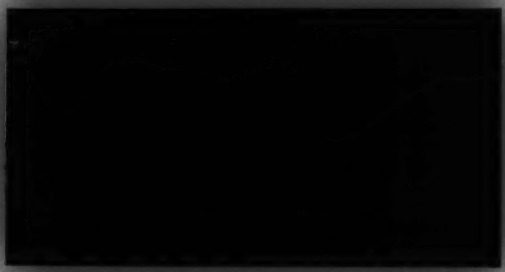

Fig. 1. Cat. Decerebrate. Stimulate left central vagus 25 milliamp. in primary; stimuli are approximately of equal duration. 1st, 8 double shocks per second. 2nd, 2 d.s.p.s. 3rd, 50 d.s.p.s. Time in 2 sec. The slight fall of blood-pressure which precedes the second stimulus is due to slight pulling on the nerve during the adjustment of the electrodes

was stimulated by means of fixed electrodes. It is interesting to note, too, that a hot swab applied to the depressor nerve often produces a marked reflex fall of blood-pressure, the thermal stimulus apparently being a very effective one.

"*Splanchnic preparation.*" It has been shown by Bayliss(5, 6, 7) that the dilator fibres in the sacral autonomic and the antidromic vasodilator fibres in the posterior nerve roots are involved in the depressor

reflex. The recent work of Lewis and his co-workers (8) suggests that these latter dilator fibres cause the liberation of a "histamine-like" substance in the skin which accumulates under prolonged peripheral stimulation and is but slowly dissipated when stimulation is discontinued. Many experiments have therefore been carried out on animals in which the circulation was restricted to the thorax and abdomen ("splanchnic animal") by means of the following procedure. The femoral, subclavian and carotid arteries on both sides are tied and the skin is completely removed from the trunk, neck and the proximal parts of the limbs. A well-marked depressor reflex can still be obtained; further, the fall of blood-pressure noted in the splanchnic preparation may not be appreciably less than that previously obtained with similar stimulation in the intact animal. This observation shows that the depressor reflex affects the splanchnic area to an important extent.

The splanchnic blood vessels are supplied by many vaso-constrictor fibres; though vaso-dilators are present, their number is small and they can only be demonstrated with difficulty (9, 10, 11). In the splanchnic animal, therefore, the fall of blood-pressure which results from depressor stimulation may be attributed largely to central inhibition of vaso-motor tone.

Results.

Frequency of stimulation,

A. *Intact animal.*

The results obtained on varying the frequency of stimulation depend to a considerable extent on the strength of current which is employed.

1. (*a*) With weak currents, increasing the frequency of stimulation may increase both the extent and the rate of the fall of blood-pressure (Fig. 1). In this figure the duration of the recovery of the blood-pressure to normal is proportional to the extent of the fall; the slope of the recovery curve in this case is least after stimulation with 2 d.s.p.s.

(*b*) Under certain circumstances the blood-pressure does not remain down under prolonged stimulation of low intensity. In Fig. 2 stimulation was carried out on each occasion for a period of about 50 sec. With a frequency of 8 d.s.p.s. the pressure fell slowly and to a small extent but soon returned to its original level and then exceeded it in spite of continued stimulation. With 20 d.s.p.s. the pressure returned more gradually to normal during the latter part of the stimulation; with 50 d.s.p.s. there was a sharp and marked fall of blood-pressure and the pressure was maintained almost at the lowest level throughout the period of

stimulation; the return to normal was complete about half a minute after stimulation was discontinued.

Fig. 2. Cat. Chloralose. Stimulate left central vagus. 25 milliamp. in primary. Stimuli: 8 d.s.p.s.; 20 d.s.p.s.; 50 d.s.p.s. Time in sec.

2. When strong currents are employed in the primary circuit there is usually little difference in the extent and rate of the fall of blood-pressure during the period of stimulation. But in Fig. 3 there is a marked

Fig. 3. Cat. Decerebrate. 0·3 amp. in primary. Stimulate left central vagus. Stimuli: 2 d.s.p.s.; 30 d.s.p.s. Time in 2 sec. Initial blood-pressure 110 mm. Hg.

difference in the blood-pressure curve when stimulation is discontinued. With 2 d.s.p.s. after 2 sec. the blood-pressure begins to rise and is back to normal in about 40 sec.; with 30 d.s.p.s. the pressure continues to fall for 12 sec. and the return to normal occupies about 70 sec.

The response of the vagus nerve to variation in frequency of stimulation is thus seen to differ from that of mixed nerves like the great sciatic, in which, as shown by Gruber(12), a fall of blood-pressure is

obtained with slowly interrupted stimuli, and with rapidly interrupted stimuli a rise results.

B. *Splanchnic animal.*

1. In this preparation too, with weak currents, increasing the frequency of stimulation may increase the extent and rate of the fall of blood-pressure. The same result is obtained after section of the right splanchnic nerve, to exclude the vaso-dilator fibres which are present in this nerve (Fig. 4) and also after section of the left splanchnic nerve.

Fig. 4. Cat. Chloralose. Splanchnic preparation. Right splanchnic nerve divided. Stimulate left central vagus. 10 milliamp. in primary circuit; secondary coil fixed throughout. Stimuli: 2 d.s.p.s.; 50 d.s.p.s. Time in 2 sec.

2. When strong currents are employed in the primary circuit it is found in the splanchnic animal (as in the intact animal) that there is little difference in the extent and rate of fall of blood when the frequency is varied. The recovery process may be prolonged, however, following the more frequent stimulation. Thus in Fig. 5 the falls of blood-pressure

Fig. 5. Cat. Chloralose. Splanchnic preparation. Artificial respiration. (Natural breathing also present.) Stimulate left central vagus. 0·3 amp. in primary. 1st stim. 20 d.s.p.s. 2nd stim. 50 d.s.p.s. 3rd stim. 10 d.s.p.s. Time in 2 sec.

which occurred as a result of stimulation with 10, 20 and 50 d.s.p.s. were 64, 64 and 70 mm. Hg respectively. The respective durations of the recovery of the blood-pressure to normal were 23, 48 and 60 sec. There was in this case an after-rise following stimulation with 10 d.s.p.s.

Pressor complications. Exceptionally it has been found that increasing the frequency of stimulation may diminish the fall of blood-pressure which occurs during stimulation and increase the extent of the after-rise.

Intensity of stimulation.

A. *Intact animal.*

Increase in the strength of the stimulus applied to the depressor nerve (the frequency being kept constant) shortens the latency of the reflex, usually increases the rate and extent of the fall of blood-pressure and may prolong the duration of the recovery process. The shortening of the latency with stronger stimulation is difficult to demonstrate conclusively in the decerebrate animal, but quite clear cut results have been obtained under chloralose (Fig. 6).

Fig. 6. Rabbit. Chloralose. Stimulate left depressor nerve. Distance of secondary coil from primary in cm. 0, 10, 15 in successive stimulations. Time in 2 sec. Initial blood-pressure 110 mm.

The other features are well shown in Fig. 7 in which all respiratory disturbances were excluded. There is an appreciable difference in the rate of the initial part of the fall when the results with secondary coil at 5 and at 2·5 are compared; the difference is very evident in the latter part of the curves. With secondary coil at 2·5 the pressure fell as much during 12 sec. stimulation as it did during 36 sec. stimulation with it at 5. With the stronger stimulus there was a marked after-fall following the cessation of stimulation.

In respect of strength of stimulation the vagus nerve also differs from the somatic nerves in the case of which the general rule is that weak

stimuli produce a reflex fall and strong stimuli a reflex rise of blood-pressure (13).

B. *Splanchnic animal.*

1. In this preparation also, increasing the strength of stimulation may increase the rate and extent of the fall of blood-pressure. Thus in a

Fig. 7. Cat. Chloralose. Artificial respiration. (Natural breathing also taking place.) Stimulate left central vagus. 1st signal: coil at 5 and coil at 2·5 for 12 sec. (superimposed tracings). The beginning and end with coil at 5 slightly precede that with coil at 2·5. Compare rate and extent of fall and the recovery process. 2nd signal: coil at 5 (36 sec.). Note in this case the more rapid return of the blood-pressure to normal after longer stimulation. Time in 2 sec.

typical experiment stimulation with coil at 22·5, 20, 15 and 12·5 cm. resulted in falls of blood-pressure of 20, 28, 50 and 54 mm. Hg respectively.

2. In some experiments there is little difference in the extent and rate of the fall of blood-pressure during the period of stimulation with different coil strengths, but a definite after-fall occurs on cessation of the stronger stimulus. In Fig. 8 the extent of the fall of blood-pressure during the period of stimulation with coil at 17, 12·5 and 7·5 was 45, 48 and 56 mm. Hg respectively. Following stimulation with coil at 17

and 12·5 recovery set in almost immediately and was complete in 30 and 37 sec. respectively; following stimulation with coil at 7·5 there was

Fig. 8. Cat. Splanchnic preparation decerebrate. Natural breathing and artificial respiration. Stimulate left central vagus for 10 sec. with coil at 12·5 and 7·5 (1st signal) with coil at 17 (2nd signal). (The stimulus with coil at 7·5 was applied first and was followed after an interval by weaker stimuli.) Blood-pressure was lowered as a result of traction of nerve prior to stim. coil at 12·5. Note after fall and slow recovery process following stim. coil at 7·5. Time in 2 sec.

an after-fall of 34 mm. during the next 24 sec., then recovery set in and was not quite complete after another 100 sec.

3. Further points are brought out in the experiment illustrated in Fig. 9. A smaller initial rise of blood-pressure is there obtained on

Fig. 9. Cat. Chloralose. Splanchnic preparation. Artificial respiration. 1st signal: stimulate left central vagus coil at 5. 2nd signal: stim. coil at 2·5. Drum stopped for periods of 30 sec. during recovery of blood-pressure. Time in 2 sec.

increasing the strength of stimulus. With stimulation coil at 5 when stimulation was discontinued rapid recovery occurred; with stimulation coil at 2·5 not only was there a greater fall of pressure during the period of stimulation and an after-fall, but in addition the blood-pressure remained at its minimal value for several minutes and then recovery occurred extremely slowly, though it was finally complete.

The longer duration of the recovery process following stronger stimulation may be noted whether the stronger precedes the weaker stimulus or *vice versa* (Figs. 8 and 9).

Prolongation of stimulation.

A. *Intact animal.*

1. (*a*) Prolongation of the period of stimulation may increase the extent of the fall of blood-pressure in animals under chloralose and in the decerebrate condition (Fig. 10, see also Fig. 7). These effects may be

Fig. 10. Rabbit. Chloralose. Stimulate left depressor nerve coil at 10. Time in 2 sec. Effect of prolongation of stimulus on extent of fall of blood-pressure and on subsequent recovery process. The slight falls which precede the stimuli are due to pulling on the nerve when adjusting the electrodes. Initial blood-pressure 105 mm. Hg.

observed with stimuli which are well below maximal, and by increasing the strength or frequency of afferent stimulation the vascular relaxation can be made to proceed much faster. The blood-pressure does not usually fall with uniform speed; there may be a rapid initial fall and the rest of the fall proceed more gradually.

(*b*) Certain variations from this rule have been encountered: (i) increasing the duration of stimulation may not increase the extent of the fall which is obtained, though submaximal stimuli are employed, (ii) the blood-pressure may tend to return to normal if weak stimulation is prolonged (Fig. 2), (iii) under continued stimulation an initial rise may pass into a fall.

2. As a rule the duration of the recovery period increases, within limits, with the length of the previous stimulation (Fig. 10).

(*a*) The recovery process may be uniformly slowed down following the longer period of stimulation. This is most often noted when little further fall of pressure occurred during the latter part of the longer stimulation. Thus in the animal from which Fig. 10 is a sample it was found at a later stage of the experiment that stimulation for 25 or 52 sec. produced falls of blood-pressure of almost equal extent; but the durations of the recovery processes were 40 and 120 sec. respectively. These results may be obtained whether the longer stimulus succeeds the shorter or *vice versa*.

(*b*) In other experiments, though the total duration of the recovery process is greater after more prolonged stimulation, the following feature is noted. After a short stimulus the blood-pressure returns rapidly to normal, while after longer stimulation a rapid partial recovery takes place followed by a gradual completion of the return of blood-pressure to normal.

(*c*) Exceptionally the recovery period may be shorter after the stimulus of longer duration (Fig. 7).

B. *Splanchnic animal.*

In this preparation too, prolongation of the period of stimulation may increase the extent of the fall of blood-pressure and the duration of the recovery process. Thus in Fig. 11 stimulation for 8 sec. lowered the

Fig. 11. Cat. Chloralose. Splanchnic preparation. Stimulate left central vagus 25 milliamp. in primary circuit. 50 d.s.p.s., secondary coil fixed. Time in 2 sec. Effects of prolongation of stimulus.

pressure by 34 mm. Hg, and recovery was complete in another 20 sec. Stimulation for 24 sec. lowered the pressure by 60 mm. Hg, and the

recovery process occupied 1 min. Later in this experiment stimulation for 50 sec. lowered the blood-pressure by 80 mm. Hg, and recovery occurred in 2 min.; 10 sec. stimulation lowered the pressure by 35 mm. Hg, and recovery took place in 24 sec.

Discussion.

1. The results described show that there is a general resemblance between the effects of increasing the frequency and increasing the strength of stimulation. Both these procedures lead as a rule to an increase in the rate and extent of the fall of blood-pressure, and under certain circumstances to a prolongation of the duration of the recovery process. Increasing the duration of stimulation may also increase the extent of the fall of blood-pressure and the duration of the recovery process. Certain exceptions to these generalisations have been described, but these may possibly be attributed to some extent to a variable admixture of pressor fibres in the nerves stimulated. Very similar results have been obtained in the decerebrate animal and in chloralose anæsthesia, and most of the observations which were made in the intact animal have also been repeated in the splanchnic preparation. The results obtained resemble in a general way those described by Liddell and Sherrington(14, 15) in their studies of the skeletal muscle reflexes. In their researches, however, the reacting tissue is very rapid in its responses and a highly sensitive optical myograph was employed. The arteriolar wall on the other hand is a much more slowly reacting tissue, and the method employed for recording the changes which take place in it is relatively clumsy and possessed of considerable inertia. Further, the vasodilatation produced may set up secondary changes in the circulation such as reducing the venous return and interfering with the nutrition of the heart.

It might be thought that these difficulties would exclude the possibility of any light being thrown by a study of these vascular reflexes on the intricate changes which take place in the nerve centres. Sharpey-Schafer(4), however, has shown that the venous return is not diminished as a result of depressor stimulation, but may be inferred to increase. It is probable too that unless the mean blood-pressure is reduced very considerably and for a long time that the nutrition of the heart is not seriously impaired. The fact that these resemblances between skeletal and vascular reflexes can be shown suggests that the processes which take place in the sympathetic centres, controlling as they do slowly reacting peripheral structures, are much slower than those in the centres controlling skeletal muscle.

2. It has been shown that increasing the frequency of stimulation often multiplies the size of the immediate effect produced (Figs. 1, 2). If it be accepted that in the splanchnic animal the vaso-dilatation observed on depressor stimulation is mainly due to inhibition of vaso-constrictor tone, then the fact that the results have been repeated in this preparation suggests that increasing the frequency of stimulation may result in the more rapid and more extensive formation of an inhibitory agent in the nerve centres; support is given to this contention by the fact that similar results are also obtained after section of the right splanchnic nerve (Fig. 4).

3. As the greater degree of dilatation which may result from increasing the duration of stimulation has been observed in the splanchnic animal it cannot be attributed wholly to peripheral accumulation of vaso-dilator substances, nor is it due to the properties of the slowly reacting peripheral mechanism. It is suggested that the process which is taking place is identical with the "inhibitory recruitment" described by Liddell and Sherrington, *i.e.* that prolongation of stimulation results in an increasing number of neurones being brought under the influence of the inhibitory process.

4. The results illustrated in Figs. 3, 7 and 10 seem to show that the duration of the return of the blood-pressure to normal does not depend entirely on the special properties of the blood vessels, for with approximately equal amounts of vascular dilatation the duration and character of the recovery process vary with the frequency, intensity or duration of the stimulation which has been previously employed. This suggests that the agency responsible for the vaso-dilatation is capable of cumulation and that it may outlast the period of stimulation. In the intact animal the site of cumulation and persistence of the dilator agent might be central or peripheral or both; as some of these observations have been repeated in the splanchnic animal (Figs. 5, 8, 9) it is suggested that the inhibitory agent may persist in the nerve centres for a length of time which depends on the amount which has been accumulated during the stimulatory period, and that it may thus retard the return of the blood vessels to their normal posture, *i.e.* a process analogous to the "inhibitory after-discharge" of Liddell and Sherrington.

Summary.

(1) The fall of blood-pressure which results from stimulation of the central end of the vagus (cat) or of the depressor (rabbit) has been studied from the standpoint of the physiology of reflex action, and particularly in the light of Sherrington's "humoral" theory.

(2) The general features of the depressor reflex are the same in the intact animal as in the animal with the circulation restricted to thorax and abdomen ("splanchnic preparation"); in the latter the fall of blood-pressure which is obtained is probably chiefly the result of inhibition of vaso-motor tone.

(3) The depressor reflex is often readily elicited by mechanical or thermal stimulation both under chloralose and in the decerebrate animal.

(4) Increase in the frequency of afferent stimulation usually intensifies the inhibitory effect produced; there is an increase in the rate and extent of the fall of blood-pressure and sometimes in the duration of the recovery of the blood-pressure to normal. Similar results have been obtained in the "splanchnic animal." The first two results have also been observed after section of both splanchnic nerves in the splanchnic preparation.

(5) Increase in the intensity of afferent stimulation in the intact and splanchnic animal increases the rate and extent of the fall of blood-pressure and may prolong the duration of the recovery period. In the intact animal it may shorten the latent period.

(6) The effect of prolongation of the period of stimulation on the extent of the fall of blood-pressure and on the duration of the recovery period in both intact and splanchnic animal is described.

(7) The results described resemble in many respects those obtained with skeletal reflexes. It is suggested they offer evidence that the processes of "recruitment" and "after-discharge" may take place in the vaso-motor "centre."

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A NOTE ON THE ACTION OF INSULIN IN NORMAL PERSONS.

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Although a very large amount of research has been devoted to the action of insulin, the literature contains remarkably little information about the action of this substance upon normal human subjects. Hence it seems worth while to put upon record the few observations described below. These were made with two objects: (*a*) insulin has been advocated as a means of treatment in cancer, on the ground of alleged beneficial results in tumour-bearing animals; it is therefore desirable to know whether it is a practicable remedy in non-diabetic persons; and (*b*) insulin is stated (Viale and Castagma(1)) to lessen the output of oxalic acid, and might provide a means of treatment for a serious affection, namely oxaluria and the recurrent formation of oxalate calculi.

Our experiments were made upon a healthy male, weighing 78 kilos, and taking ordinary diet. The course of the two chief experiments in which 15 units, and 11·5 units, of insulin were taken is given in tabular form below (Table I).

Table I. Percentages refer to blood sugar.

10. v. 27		29. viii. 27	
8.30 a.m.	Breakfast	8.30 a.m.	Breakfast
10.45 „	0·085 p.c.	11.0 „	0·093 p.c.
11.5 „	Insulin 15 units	11.10 „	Insulin 11·5 units
12 noon	Sweating. Some tremor	11.45 „	Hungry. No sweating till 12.30
12.20 p.m.	0·051 p.c.	12.30 p.m.	0·040 p.c. Tremor, sweating, very hungry
12.40 „	Sweating and tremor increased. Very hungry	12.45 „	Blood films taken (see Table II)
1.0 „	0·044 p.c.	1.40 „	0·040 p.c.
1.5 „	Lunch	1.45 „	Still some sweating and tremor. Very hungry. Lunch
4.30 „	0·11 p.c.	3.30 „	0·096 p.c.

In a preliminary experiment the injection of 7 units of insulin was followed by some sweating and a feeling of muscular weakness. Fortunately the descriptions given by Maclean and by Lublin, which are quoted below, had not been read, so that the effects experienced cannot

be attributed to suggestion. Larger amounts (11·5 or 15 units) produced (1) profuse sweating, which begins from one to one-and-a-half hours after the injection; (2) muscular weakness and tremor, which are sufficient to make writing rather difficult; and (3) extreme hunger. The interest of this group of symptoms seems to be that the condition and sensations are exactly similar to those brought about in an untrained person by prolonged and exhausting muscular exercise. Probably both states depend upon the same cause, namely, lack of available carbohydrate. The onset of great hunger after injection of insulin, in a person who has taken the usual quantities of food, and less than the usual amount of exercise, is rather surprising, and is of interest in connection with the physiology of hunger. Here the comparison with the effects of exertion in an untrained person is of especial interest, for the ability to take vigorous exercise, *e.g.* hill-climbing, for many hours without any desire for food is one of the best signs of fitness. It would be of some interest to know whether insulin would have much less effect upon the same person when well trained. After a large midday meal all the symptoms ceased, but fatigue lasted throughout the day, and this offers another point of resemblance to the effects of exercise.

There was no distinct difference between the action of 11·5 and of 15 units, but 7 units had very much less effect. In the last experiment a differential count was made of 500 white cells in blood taken when the symptoms were most distinct. A control observation was made on another day at a time having the same relation to meals (Table II).

TABLE II.

	29. viii. 27 11.10 a.m. Insulin 11·5 units 12.45 p.m. Blood films taken	1. ix. 27 Control 12.45 p.m.
Leucocytes per cub. mm.	7300	7100
Neutrophils	47 p.c.	66 p.c.
Eosinophils	3 „	2 „
Basophils	2 „	1 „
Monocytes	7 „	11 „
Lymphocytes	41 „	20 „
In vitally stained films reticulocytes (as percentage of red corpuscles)	3·5 p.c.	0·5 p.c.

The results show that under the action of insulin there is an absolute decrease in neutrophil leucocytes, and an absolute increase in lymphocytes. A blood picture of this kind is seen in exophthalmic goitre, in which condition likewise there is an increase in vitally stained red corpuscles (reticulocytes).

A number of estimations of oxalic acid were made, but the methods used (Dakin[2], Wegrzynowski[3]), were not found sensitive enough for the very small amounts in the urine of a few hours only, and we were unable to decide whether there was any alteration in the hourly rate. But in any case, if such amounts of insulin had to be taken, the treatment would be quite impracticable for oxaluria.

Maclean[4] describes the effects of 20 units of insulin in two healthy men who had taken 50 grm. of sugar by the mouth. In one subject there were no symptoms; in the other there was profuse sweating of the face, scalp and neck, weakness, inability to perform fine movements, and dizziness, although the blood sugar was not observed to fall below 0·07 p.c. Lublin[5] examined the respiratory exchange of three normal persons after injections of insulin in amounts up to as much as 30 units, and apparently did not observe any of the effects described here. But the records of his experiments show that: (1) considerable amounts of sugar (20 to 100 grm. levulose or dextrose) were taken after the insulin in every case; (2) that the blood sugar was not lowered very much, the greatest reductions recorded being from 0·081 to 0·068, and from 0·108 to 0·092 p.c.; and (3) that the subjects were at rest in bed throughout. Lublin seems to have noted extreme hunger ("Heisshunger") and some tremor ("Der eine...gab an, etwa 1 Stunde nach der Mahlzeit fast regelmässig ein Zittern am ganzen Körper verspürt zu haben") in an under-nourished patient to whom 10 units of insulin were given before meals as a means of treatment, but the account (*loc. cit.* pp. 113–114) is not very clear.

Summary.

Injection of insulin in a normal person was followed by sweating, muscular weakness and tremor, hunger, and changes in the differential blood count.

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THE RESPIRATORY QUOTIENT AFTER EVISCERATION IN CATS.

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THE evidence for the Hill-Meyerhof hypothesis that carbohydrate is "the primary fuel of muscles" comes largely from studies of the respiratory quotient. In 1910 Porges(1) and Porges and Salomon(2) demonstrated that ligation of the abdominal aorta and inferior vena cava just below the diaphragm causes the respiratory quotient, in both normal rabbits and depancreatised dogs, to rise to the neighbourhood of unity. Since the anterior preparation they used is essentially a muscle preparation, their work has been cited as evidence in support of the theory that muscle can utilise carbohydrate only as a source of energy. Meyerhof(3), from a study of the respiratory quotient of isolated frog's muscle, makes a similar claim. Furusawa(4) states that the excess respiratory quotient of man due to short periods of exercise is unity, and hence "carbohydrate only is responsible for the process of contraction and recovery from it." Further evidence in favour of this view was produced by Burn and Dale(5), who showed that the respiratory quotients of decapitated, eviscerated cats, kept alive by artificial respiration and slowly infused with a solution of glucose, is in the neighbourhood of unity. They assumed this to represent the respiratory quotient of muscle, plentifully supplied with carbohydrate, since it is obtained in a preparation consisting essentially of muscle, bone, heart, lungs and skin. Furthermore, it is not affected by the administration of insulin, and it is the same in previously depancreatised (diabetic) cats as in normal cats.

Much, however, of this evidence, which underlies the assumption that muscle tissue can utilise carbohydrate only as a source of energy, has been challenged. In 1912 Murlin, Edelmann and Kramer(6) showed that the rise in the respiratory quotient obtained in the experiments of Porges and of Porges and Salomon was accompanied by a simultaneous reduction in the CO_2 content of the blood, probably due to overventilation from increased respiration or overaeration from increased blood flow through the lungs, following the clamping of the abdominal

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vessels. They concluded that "the experiments of Porges and of Porges and Salomon have no bearing on the problem of the oxidation of sugar." Takane(7), working with strips of rat's diaphragm suspended in serum, found in four observations an average respiratory quotient of only 0·79. Doisy and Bechmann(8) determined the respiratory quotients of the hind limbs of 17 dogs by comparing the gases of the arterial and venous blood from the femoral vessels. Two only showed a respiratory quotient of unity. The average of 14 results falling within the normal limits of 0·67 and 1·05 was 0·80. In this laboratory Markowitz(9) has shown that the respiratory quotient of eviscerated dogs is less than unity, averaging about 0·90, an observation which has been confirmed by Soskin(10), although the latter occasionally obtained a respiratory quotient of 1·0. While this work was in progress, Himwich and Castle(11) published a paper showing that the respiratory quotient of resting mammalian muscle *in situ*, as determined by a study of the gases of the blood entering and leaving a muscle in the hind limb of the dog, is close to that of the body as a whole, and hence "resting muscle under normal conditions oxidises besides carbohydrates either fat or protein or both, probably in the same proportions as does the rest of the body." In 1925 Henderson and Haggard(12) concluded from observations on the respiratory quotients of trained athletes "that in whatever proportion fat and sugar are being burned during rest just before the exercise, they are burned in nearly the same proportion to produce the energy for doing work or for the recovery process in the muscles." Later, Wilson, Rivkin, Levine and Berliner(13) were also unable to confirm the work of Furusawa, for they found, in experiments with children, that the respiratory quotient of the extra metabolism due to moderate exercise is always less than unity. Still more recently Rapport and Ralli(14) have likewise failed to obtain a respiratory quotient of unity for the excess metabolism of exercise in dogs.

A survey of the above observations indicates that, aside from Meyerhof's work on isolated frog's muscle, the work of Burn and Dale remains as the most direct evidence in support of the theory that carbohydrate is the sole fuel of muscle. It was, therefore, decided to investigate further the respiratory quotients of decapitated and eviscerated cats, in the hope of finding a cause for this discrepancy.

Methods.

Cats which, unless otherwise stated, had been starved for 24 hours, were used in all experiments. Decapitation was performed under ether

anæsthesis, and artificial respiration was at once established by means of the respiration pump described by Taylor and Olmsted(15). After an interval of from 30 minutes to 1 hour, the respiratory quotient was determined by analysis of a sample of expired air, taken from a rubber bag, having a capacity of about 13 litres, attached to the outlet of the pump. Air was collected in the bag for periods of 15 minutes for each determination, and the volume was measured by passing through a metre. These measurements showed the minute volume of the pump to remain practically constant for each experiment, with an average value of about 720 c.c. A rate of 18 to 19 strokes per minute was maintained throughout. In later experiments in which the blood CO_2 was simultaneously determined, blood samples were collected from the carotid artery, and the CO_2 content determined by the method of Van Slyke and Neill(16). Following usually two determinations of the respiratory quotient, or respiratory quotient and CO_2 content of the arterial blood, the preparation was eviscerated. The stomach, intestines, spleen and pancreas were removed after ligaturing all necessary blood vessels. The arteries were always tied first in order to reduce the loss of blood to a minimum. The liver was left *in situ*, but since the hepatic artery and portal vein had been divided between ligatures, it remained a mere *cul de sac* on the vena cava, and presumably had but little effect on the metabolism of the remainder of the preparation. The abdominal cavity was then filled with warm Locke's solution, and the abdomen closed. The preparation was fixed upon an electrically heated table and covered with towels. Its temperature was determined by means of a thermometer inserted through a skin incision on the chest into the loose tissue of the axilla, and was controlled by turning on or off one or more heating lamps under the metal table top.

Experimental results.

Table I shows that evisceration consistently causes the respiratory quotient to rise, usually to the neighbourhood of unity. The observation

Table I.

Cat No.	R.Q. before evisceration	R.Q. after evisceration				
		1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.
1.	0·79 0·80	0·90 0·86	0·99 0·86	—	—	—
2.	0·74 0·72 0·74	1·04 0·94	1·13 1·20	1·29 1·62	—	—
3.	0·71 0·73	0·98 0·92	0·84 1·05	1·34	—	—
4.	0·71 0·79	0·93 0·97	—	1·23	1·07	1·07
5.	0·78 0·80	0·94 0·94	1·00 0·97	1·25	—	—
6.	0·72	1·80 1·03	1·04	1·05	0·87	—

Respiratory quotients of decapitate cats before and after evisceration.

TABLE II.

Cat 1. Weight, 3·0 kg.

	Vol. air breathed per hr. dry and at 0° C. and 760 mm. press. (c.c.)	Period of collection of air after evisceration (min.)	CO_2 in expired air (p.c.)	O_2 absorbed (p.c.)	CO_2 expired. Total vol. per. hr. (c.c.)	O_2 absorbed. Vol. per hr. (c.c.)	Metab. rate of normal (p.c.)	CO_2 production (calculated from R.Q. before evisceration) (c.c.)	Excess CO_2 (c.c.)	R.Q.	Blood sugar (p.c.)	Glycogen content of muscles of hind leg (p.c.)
Before evisceration. Aver. of 2 samples	38400	—	2·06	2·57	790	986	100	790	—	0·80	0·153	0·46
1st hr. after evisceration. 2 samples	37100	24–39	1·30	1·47	404	460	47	371	33	0·88	—	—
		49–64	0·87	2·01								
		Average	1·09	1·24								
2nd hr. after evisceration. 2 samples	36600	71–86	1·09	1·10	384	414	42	332	52	0·93	0·097	0·42
		106–121	1·01	1·17								
		Average	1·05	1·13								
		Total excess CO_2 (2 hr.)			...	...	...	...	...	...	85	
				Cat 2. Weight, 2·7 kg.								
Before evisceration. Aver. of 3 samples	36200	—	1·80	2·40	652	869	100	652	—	0·73	0·270	—
1st hr. after evisceration. 2 samples	38200	8–23	1·44	1·38	523	527	61	398	125	0·99	—	—
		55–50	1·29	1·37								
		Average	1·37	1·38								
2nd hr. after evisceration. 2 samples	38600	71–86	1·13	0·97	428	363	42	274	154	1·17	—	—
		104–119	1·09	0·90								
		Average	1·11	0·94								
3rd hr. after evisceration. 2 samples	Not measured, taken as 38600 (3 hr.)	132–147	0·93	0·72	320	228	25	163	157	1·45	0·139	—
		154–169	0·72	0·46								
		Average	0·83	0·59								
		Total excess CO_2 (3 hr.)			...	...	...	...	...	...	436	
				Cat 3. Weight, 3·2 kg.								
Before evisceration. Aver. of 2 samples	40700	—	2·71	3·76	1102	1534	100	1102	—	0·72	0·273	—

				Cat 3. Weight, 3·2 kg.								
Before evisceration. Aver. of 2 samples	40700	—	2·71	3·76	1102	1534	100	1102	—	0·72	0·273	—
1st hr. after evisceration. 2 samples	40300	10–25	1·76	1·82	673	709	46	507	166	0·95	—	—
		45–60	1·57	1·71								
		Average	1·67	1·76								
2nd hr. after evisceration. 2 samples	41000	76–81	1·35	1·65	537	590	38	419	118	0·94	—	—
		105–120	1·27	1·23								
		Average	1·31	1·44								
3rd hr. after evisceration. 1 sample	40000	170–185	1·10	0·82	440	328	21	231	209	1·34	0·163	—
		Total excess CO_2 (3 hr.)							493			
				Cat 4. Weight, 1·95 kg.								
Before evisceration. Aver. of 2 samples	40200	—	2·24	2·99	900	1202	100	900	—	0·75	—	0·19
1st hr. after evisceration. 2 samples	40600	1–16	1·41	1·52	520	548	46	414	106	0·95	—	—
		29–44	1·15	1·18								
		Average	1·28	1·35								
2nd and 3rd hr. after evisceration. 1 sample	83800	149–165	0·84	0·68	704	570	24	432	272	1·23	—	0·20
		Total excess CO_2 (3 hr.)							378			
				Cat 5. Weight, 4·83 kg.								
Before evisceration. Aver. of 2 samples	41700	—	2·68	3·38	1118	1410	100	1118	—	0·79	—	—
1st hr. after evisceration. 2 samples	41400	5–20	2·18	2·32	907	965	68	760	147	0·94	—	—
		39–54	2·19	2·33								
		Average	2·19	2·33								
2nd hr. after evisceration. 2 samples	41600	78–93	2·06	2·05	849	857	61	682	167	0·99	—	—
		102–122	2·01	2·06								
		Average	2·04	2·06								
3rd hr. after evisceration. 1 sample	41900	160–175	1·47	1·18	620	495	35	392	228	1·25	—	—
		Total excess CO_2 (3 hr.)							540			
		Cat 6. Weight, 3·25 kg. Vol. air not reduced to standard conditions.										
Before evisceration. 1 sample	40800	—	2·26	3·02	932	1232	100	932	—	0·72	—	0·31
1st hr. after evisceration. 1 sample	41600	37–52	1·47	1·45	620	603	49	457	163	1·03	—	—
2nd hr. after evisceration. 1 sample	41600	94–105	1·25	1·20	520	499	40	373	147	1·04	—	—
3rd hr. after evisceration. 1 sample	41600	163–178	1·13	1·08	470	448	36	336	134	1·05	—	0·33
		Total excess CO_2 (3 hr.)							444			

of Burn and Dale that the respiratory quotient of the decapitate, eviscerated cat is about 1·0 is thus confirmed.

However, it seemed desirable to investigate other possible causes for this sudden rise in the respiratory quotient, before assuming that it indicated a change to a purely carbohydrate type of metabolism upon removal of the viscera. Obviously, it could not be due to overventilation in the ordinary sense, since the rate and minute volume of the respiration pump remained constant. On the other hand, a relative increase in ventilation might be thought of as a factor, since the CO_2 production is considerably reduced by evisceration, while no corresponding reduction in ventilation takes place. It has been shown by Hunt and Bright(17) that the abdominal viscera account for about 40 p.c. of the metabolism. Our observations show that the reduction in O_2 consumption during the first hour after evisceration varies from 32 to 54 p.c., but the metabolic rate continues to fall as long as the animal lives. Table II shows in detail for a series of cats the marked changes which occur in the R.Q., CO_2 output and O_2 intake following evisceration. The total respiratory exchange is reduced, but the reduction in O_2 absorption is relatively greater than that in CO_2 elimination. Hence the higher respiratory quotients.

It now remained to discover, if possible, the source of the excess CO_2 eliminated. The CO_2 content of the blood was, therefore, determined in four cats before and after evisceration. Table III shows the results obtained.

TABLE III.

Cat No.	Weight (kg.)		Before evisceration		After evisceration 1st hr.	2nd hr.	3rd hr.	4th hr.	Remarks
7	2·2	R.Q.	1·09	1·05	1·80	—	—	—	Stomach
		Blood CO_2 vol. p.c.	32·71	32·89	10·11	—	—	—	full
8	1·8	R.Q.	1·01	1·00	1·35 1·15	—	—	—	Stomach
		Blood CO_2 vol. p.c.	33·21	32·92	25·29	16·20	6·66	—	full
9	2·5	R.Q.	0·81	0·85	0·95	1·08	0·90	0·96	
		Blood CO_2 vol. p.c.	32·09	32·62	21·58	18·45	15·28	11·67	
10	3·5	R.Q.	0·80	0·93	1·00	1·09	0·99	1·02	
		Blood CO_2 vol. p.c.	20·50	20·89	17·40	14·90	11·35	9·37	

It is evident that a very marked decrease in the CO_2 content of the arterial blood takes place following evisceration, and the question arises as to whether the CO_2 thus lost from the blood and tissues is sufficient

to account for the rise in the respiratory quotient. That such may be the case is indicated by the results obtained with cats Nos. 7 and 8. Both of these animals had been fed shortly before the commencement of the experiment, and their stomachs were found to be full at the time of evisceration. In spite of the resulting high initial respiratory quotients removal of the viscera caused marked increases, the values obtained being explicable only on the assumption that CO_2 was being removed from the blood by some abnormal mechanism. The correspondingly low blood CO_2 values prove this to have been the case.

Table II shows that in the three hour period following evisceration an average of 458 c.c. of CO_2 over and above the calculated production was obtained from five cats (Nos. 2 to 6) averaging 3·18 kg. in weight. Assuming an average weight of 2·0 kg. after evisceration this means that about 230 c.c. per kilogram of preformed CO_2 were washed out from an animal's body in three hours. These calculations are based upon the assumption that no change in the type of metabolism is caused by the removal of the viscera, *i.e.* that the *actual* respiratory quotient remains constant at its initial value of 0·72 to 0·80. If one assumes a shift to a purely carbohydrate type of metabolism to occur on evisceration with a resulting respiratory quotient of unity, it would, of course, be unnecessary to account for the washing out of such a large amount of preformed CO_2. Nevertheless, the amount found is comparable with that obtained in one experiment in which overventilation alone was applied to a normal cat under amytal anæsthesia, so that the assumption that evisceration causes a change in the type of metabolism becomes unnecessary.

The results of this experiment are given in Table IV. This shows that an excess of 579 c.c. CO_2 over and above the calculated production was blown off during the two hours of overventilation. It should be pointed out that the actual excess was probably less than this, since the percentage of CO_2, upon which the calculation of the total CO_2 during the first hour depends, is taken as the average of samples of expired air collected during the first five minutes, and forty minutes later. There is no doubt that the percentage of CO_2 falls much more rapidly at the start of overventilation than later on, so that it would probably have been more correct to take the forty minute sample. If we do this the excess production becomes 253 c.c., instead of 434 c.c., and the total excess 398 c.c. This cat weighed 2·8 kg. and the blood CO_2 fell during the overventiliation from 40·3 to 22·2 vols. p.c., roughly 18 vols. p.c. If we suppose that all the water of the body had a CO_2 capacity equal to that

TABLE IV.

Overventilation.

(Cat *A*. Weight, 2·8 kg.)

Pump rate increased from 19–64 strokes per min.

	Vol. expir. air per hr. dry 0° C. 760 mm. c.c.	Time after changing rate of pump min.	CO_2 in expired air p.c.	O_2 absorbed p.c.	CO_2 vol. expired c.c.	O_2 vol. absorbed c.c.	Metab. rate p.c. of norm.	CO_2 calculated produc. c.c.	CO_2 excess c.c.	R.Q.	Blood CO_2 vol. p.c.
Norm. vent. 100–120 min. after decapitation	36360	—	2·65	3·61	963	1313	100	963	—	0·73	40·32
1st hr. of overvent. 2 samples	121140	0·5– 5·5	0·96	0·64	993	763	58	559	434	1·50	27·70
		39 –45·5	0·67	0·62						1·08	
		Average	0·82	0·63					Average	1·29	
After 2 hr. overvent.	97980	99 –105·5	0·56	0·56	549	549	42	404	145	1·00	22·21
		Total excess CO_2 in 2 hr.			...	...	...	...	579		

NOTE. A further sample of air taken 224 to 231 min. after commencing overventilation showed the R.Q. to be 0·76, the blood CO_2 to be 23·07 p.c., the metabolism to be 52 p.c. of that before starting the overventilation, and the calculated volume of CO_2 produced to be equal to the amount found in the expired air.

of blood, and in the course of the experiment lost as much CO_2 as the blood, then, since water forms roughly two-thirds of the body weight ($2{\cdot}800 \times \frac{2}{3} \times 18$), 336 c.c. of CO_2 would be accounted for.

It remains to be seen whether prolonged excessive overventilation may not cause the removal of CO_2 from the bones, and this possibility is at present under investigation in collaboration with Laurence Irving.

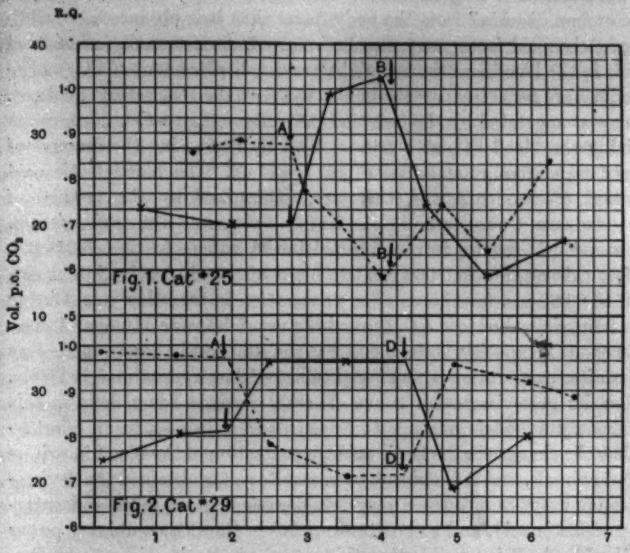
Krogh(18) has also shown that from a rabbit weighing 1800 grm. at least 250 c.c. of CO_2 can be washed out from the body, in excess of the production, by artificial ventilation of the lungs during 1·5 hours, or about 140 c.c. per kg. in 1·5 hours. This caused the respiratory quotient to rise to 1·80.

It is thus seen that overventilation alone, if carried to an extreme degree, can cause the washing out of a sufficiently large volume of CO_2 to explain most of the results shown in Tables I, II and III.

In this connection it is interesting to note the marked fall in O_2 consumption which accompanied the overventilation. This may possibly be related to a lowered blood-pressure, which Dale and Evans(19) have shown to occur when the CO_2 tension in the blood is reduced.

An effort was next made to restore the respiratory quotient to its initial value by again increasing the CO_2 content of the blood. This was done in two ways. In the first attempt, after the typical fall in blood CO_2 and increase in respiratory quotient following evisceration had been obtained, the minute volume of the respiration pump was reduced.

Its rate was left constant. The results for one of the two cats used are shown in Fig. 1. Since this procedure resulted in a considerable degree of anoxæmia, in a second experiment a Douglas bag containing expired air, with a CO_2 content of 3·43 p.c. and O_2 content of 17·44 p.c., was



Dotted line = bl. CO_2. Solid line = R.Q. *A* = eviscerated. *B* = lessened ventilation. *D* = breathing expired air.

attached to the intake of the pump. The results are shown in Fig. 2. It will be seen that in both cases evisceration caused a typical rise in the respiratory quotient to about unity accompanied by a fall in the CO_2 content of the blood. But when the blood CO_2 was restored to something like its normal value the respiratory quotient again fell, although a tendency for the blood CO_2 to decrease again and the respiratory quotient to rise will be noticed. It seems fairly evident that the respiratory quotient of the decapitate, eviscerated preparation can be made to vary at will by allowing the CO_2 content of the blood to vary, and that the latter steadily decreases unless special precautions are taken to maintain it at a normal level.

Two explanations, at least, are possible to account for this falling CO_2 content of the blood. So far we have considered only the mechanical factor. We have shown that when the CO_2 production is reduced by evisceration and the volume of air ventilating the lungs is allowed to remain constant, a relative overventilation occurs which "washes out the carbon dioxide" from the body faster than it is produced. In addition a chemical factor may also be involved. An accumulation of fixed acids in the blood and tissues might cause a displacement of CO_2 and so produce an artificial increase in the value of the respiratory quotient. This was suggested by the fact that difficulty was experienced in maintaining the blood CO_2 at a normal level by decreasing the amount of ventilation after evisceration (Figs. 1 and 2). The blood lactic acid content of two cats was, therefore, determined by the method of Friedmann, Cotonio and Schaffer(20) before and after evisceration. It was found to rise from a normal initial value of about 0·015 p.c. before evisceration to 0·060 p.c. two hours after evisceration in one cat, and to 0·067 p.c. four hours after evisceration in the other cat. During the same time the blood CO_2 showed the usual decrease. In one of these cats the CO_2 capacity of the blood was simultaneously determined. One hour after evisceration it was found to have fallen approximately 25 p.c. below its pre-evisceration value. Further work is being done in this laboratory in collaboration with Soskin and Thomas, in an effort to follow the changes in the lactic acid concentration of the blood in eviscerated animals, and it is evident from results so far obtained that evisceration in every case causes a very considerable increase in the concentration of lactic acid, and a simultaneous fall in the CO_2 combining power of the blood. It is possible that this increase in lactic acid may be due, in part at least, to the overventilation, since Anrep and Cannan(23) have found that formation of lactic acid occurs in a heart-lung preparation when it is overventilated.

Discussion.

On the basis of finding a high respiratory quotient in decapitated and eviscerated cats, Burn and Dale(5) cautiously concluded that "it is quite possible that such a quotient...may indicate the use by muscle of dextrose alone as its source of energy requirements." This conclusion has been widely quoted as evidence in support of the Hill-Meyerhof hypothesis that carbohydrate is the primary fuel of muscle. It has been assumed that the eviscerated animal, being essentially a muscle preparation, is incapable of utilising other sources of energy, and so a shift

to a purely carbohydrate type of metabolism occurs with a resultingly high respiratory quotient. But the experiments recorded in this paper show that with the rise in value of the respiratory quotient a simultaneous fall in the CO_2 content of the blood occurs. This fall is believed to depend upon two factors: (1) a mechanical overventilation which causes a washing out of preformed CO_2, and (2) a chemical factor which results in the displacement of a considerable volume of CO_2 from the blood and tissues by reason of an increased concentration of lactic acid. These two factors are probably sufficient to account for the increased amount of CO_2 eliminated after evisceration, and so make it unnecessary to assume that any shift to a purely carbohydrate type of metabolism occurs. The source in the tissues of the extra CO_2 which is pumped out in these experiments is at present being investigated in this laboratory in collaboration with L. Irving.

If the energy which is set free in an eviscerated preparation depends on oxidation of carbohydrate only, it is impossible, in our present state of knowledge, to see where most of this carbohydrate comes from. Aside from the sugar in the blood, which soon falls to a very low level in the eviscerated preparation, there is no visible carbohydrate. Best, Hoet and Marks(21) have shown that the glycogen store of the muscles is not decreased in the eviscerated animals, and this observation we have confirmed (see Table II). Soskin(22) has also demonstrated that in the absence of the liver, muscle glycogen is not an available source of blood sugar. If the muscle glycogen is not available as a source of energy, whence does the carbohydrate come?

The average eviscerated cat utilises more than two calories of energy per hour. For cats Nos. 1, 2 and 3, the blood sugar levels at the time of evisceration and at the conclusion of the experiment were determined as shown in Table II. It will be noted that an average decrease of approximately 0·04 p.c. per hour occurred. Assuming the maximum total volume of 400 c.c. for blood and tissue fluids, this means that sugar disappeared at the rate of 0·16 grm. per hour, which would account for about 0·66 calories and leave a balance of almost 1·5 calories per hour, or about three-quarters of the total amount of energy expended, to come from other sources.

It should further be noted that two of the cats used in this research had an initial respiratory quotient of unity. Evisceration in them also caused a proportionately great increase in the value of the respiratory quotient. Here there could be no question of a shift in the type of metabolism, since carbohydrate was already being utilised, and yet as

marked changes in the respiratory quotient and blood CO_2 content were observed as in other experiments. We are, therefore, compelled to conclude that the high respiratory quotient seen in decapitate, eviscerated cats is an artificial phenomenon and is not a trustworthy guide to the type of metabolism proceeding in the preparation. This does not rule out the possibility of a change in metabolism occurring on evisceration, but it does make it entirely unnecessary to postulate a shift to a carbohydrate type of metabolism in order to explain the results obtained. This type of experiment cannot, therefore, be used as evidence in favour of the Hill-Meyerhof hypothesis that muscle can utilise carbohydrate only as a source of energy.

Conclusions.

1. The respiratory quotient of the decapitate cat rises very considerably upon evisceration, but the rise is accompanied by a simultaneous decrease in the CO_2 content and capacity of the blood.

2. The respiratory quotient can again be restored to its original value by maintaining the blood CO_2 at its normal level.

3. The fall in blood CO_2 is due to at least two factors: (*a*) a relative overventilation following evisceration, due to decreased CO_2 production without a compensating reduction in lung ventilation; (*b*) a simultaneous increase in the concentration of the lactic acid in the blood.

4. Experiments based upon observations of the respiratory quotients of decapitate, eviscerated cats, artificially ventilated, can offer no evidence in support of the theory that carbohydrate is the only source of energy for muscle.

In conclusion, I wish to express my thanks to Prof. J. J. R. Macleod for suggesting this research, and for his kindly supervision throughout. My thanks are also due to Mr J. C. Thomas for estimating the lactic acid of the blood in the last two experiments, and to Miss Margaret McKay for doing the muscle glycogen determinations.

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A NON-SPECIFIC PRESSOR PRINCIPLE DERIVED FROM A VARIETY OF TISSUES.

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Introduction.

In the course of a study of the effects of extracts made from various tissues autolysed under aseptic conditions and of pancreatic digests of similar tissues, it was frequently observed that injection of certain extracts into dogs anæsthetised with amytal was followed by a rise in the blood-pressure level. This rise in blood-pressure occurred, as a rule, subsequent to a preliminary fall, if injections were made by the intravenous route, whereas intraperitoneal injections often resulted in a pure pressor effect. Such pressor effects were of particular interest because of their long duration. An effort was therefore made to prepare and fractionate tissue extracts with a view to obtaining the apparently non-specific pressor principle in a purer and more concentrated form, and to free it as far as possible from the almost universally occurring depressor principles. A general outline of the methods adopted and the preliminary physiological study of the purified extracts have been the subject matter of a preliminary report (1).

Literature.

That a large variety of tissue extracts (excepting those derived from the chromaffin system or the posterior pituitary body) may produce on intravenous administration pressor, as well as depressor effects, has long been recognised.

Tigerstedt and Bergman (2), 1898, prepared extracts of kidney tissue which caused a rise in blood-pressure when injected into the blood vessels of the rabbit. They stated that the active principle was destroyed by boiling.

Vincent and Sheen (3), 1903, published the results of an extensive investigation of the effects of extracts, prepared in different ways and obtained from various tissues, upon the blood-pressure. While the

common result obtaining, as a rule, following intravenous administration of their extracts was a depressor effect, they nevertheless emphasise that pressor substances are present in various tissues. Mixed pressor and depressor effects were frequently obtained by these authors, and in certain instances pure pressor effects were obtained. They found also that boiling tended to remove the pressor effects of their extracts, thus confirming, in a way, Tigerstedt and Bergman.

Vincent and Sheen obtained best results as far as demonstration of a pressor substance was concerned by making their extracts from absolutely fresh tissue with physiological saline solution.

They felt that their results justified the provisional suggestion that all glandular tissue, and probably all animal tissues, contain both a pressor and a depressor substance.

More recent reference in the literature to this subject has been made by Roger(4) (1922) and by James, Laughton and Macallum(5) (1926).

Very recently, Vincent and Thompson(6) (1928) have obtained evidence which has led them to postulate that under certain conditions stimulation of the intestine, muscle and skin causes an autocoid having a pressor effect to be liberated into the blood stream. In a footnote to this paper they state that they have repeated and confirmed the earlier work of Vincent and Sheen(3) on the presence of a pressor substance in many fresh tissue extracts. They add further that it is frequently destroyed by moderate elevation of temperature.

Method of preparing potent pressor extracts.

A great deal of preliminary work was done in order to ascertain the most satisfactory method of obtaining highly potent pressor extracts.

Contrary to Vincent and Sheen(3), and Vincent and Thompson(6), it was found that heating to boiling did not offer an obstacle in the further purification of the pressor extracts. Also it was early observed that the principle was soluble in acetone containing only a small percentage of water. Consequently, in the method which was finally adopted as routine in the concentration and purification of the "pressor fraction," "heat" and "acetone extraction" formed the main basis.

A method which has proved satisfactory in our hands, but which is only one of many possible modifications to arrive at the same result, is as follows:

Fresh tissue is thoroughly minced and added to five volumes of boiling water. The temperature of the mixture is quickly raised to boiling

point and maintained at this level for five minutes, the contents of the vessel being vigorously stirred. The use of acidulated water is a matter of option. Filtration of the hot mixture may be facilitated by the more thorough coagulation of the proteid in the process of heating by the use of 0·1 p.c. acetic instead of neutral distilled water as the extracting medium. The boiling mixture is filtered and the filtrate is concentrated in vacuo, at low temperature.

When the concentration process is about two-thirds completed, it is convenient to remove separated fatty substances by mechanical means and to free the extract of other lipoids by ether extraction in a separating funnel. Concentration may then be carried forward in vacuo until the extract is of the consistency of a thick syrup.

Three volumes of absolute acetone are then added and the concentrate thoroughly extracted. The acetone layer which forms contains much of the pressor fraction, and is removed. The residue is re-extracted with a further three volumes of acetone. This process may be repeated three or four times. The acetone extracts are combined and concentrated in vacuo to a thick syrupy state, and this syrup is again treated as outlined above. The whole process may be repeated once more. A watery or saline solution of the final acetone extract after all traces of acetone have been removed, makes, as a rule, a fairly satisfactory product. It does, however, contain more or less of depressor material. A further purification may be effected by obtaining the pressor fraction with adherent substances in solution in about 98 p.c. acetone. One-third volume of ether is then added and finally a trace of water, a few drops at a time, meanwhile shaking the mixture vigorously. On standing a water layer separates which contains both pressor and depressor substances, while in the acetone-ether mixture a much higher ratio exists of pressor to depressor substance.

Some physical and chemical properties of the active principle.

The pressor substance is freely soluble in absolute alcohol and insoluble in absolute acetone. Acetone containing a trace (one to three p.c.) of water is a most usual solvent. It is soluble in mixtures of alcohol and acetone, of alcohol and ether, and alcohol and petroleum ether, and of acetone, ether and water. It may be fractionally precipitated from absolute alcohol by addition of petroleum ether.

It may be removed, to a large degree, from aqueous solution by treating with charcoal (Norit). The degree of adsorption is apparently related to *p*H, and at *p*H 8 may be almost complete. It has been released

again from the adsorbent by treating with boiling water made acid to congo.

Practically complete loss of potency has occurred when the water bath instead of the vacuum still has been used to concentrate large amounts of the original watery extract. Small amounts have been concentrated by an air current with satisfactory results. The exact degree of danger which may exist when drying of extracts in the air is attempted is not yet established. It is most probable, however, that oxidation of the active principle results in loss of its physiological effect. Due precaution should therefore be taken to guard against possible oxidation.

Treatment with acetic acid anhydride caused complete loss of potency in one experiment, and no acetyl derivative was obtained.

Dilute watery extracts of material purified by repeated acetone extraction and vacuum concentration have been boiled down almost to dryness over a free flame without any great loss in potency. Also similar solutions of the pressor substance have been treated with sulphuric acid up to 5 p.c. concentration, without loss of potency. Ammonia and dilute sodium hydroxide do not appear to be harmful, even at boiling temperature.

Relation of tissue used to nature of final product.

The work herein reported has not been especially directed at obtaining maximum yields of the pressor principle. It has been the objective, rather, to demonstrate conclusively the presence of the principle in extracts of a wide variety of tissues and to obtain it in each case in as pure a form as possible. It is not possible, therefore, at this time to make comparisons on a quantitative basis of the amount of the principle which it is possible to extract from different tissues. The tissues which have been studied consist of the following: skeletal muscle, liver, kidney, spleen, ovary, testis, prostate, stomach, thymus, intestine, whole fœtus, and several commercial preparations of pepsin and pancreatin. A potent pressor extract has been obtained in each instance, with the exceptions of certain commercial brands of pepsin and pancreatin. The extract of prostate glands has certain qualities which suggest that the chief active pressor principle is in this case different from that present in extracts of different origin. This feature will be enlarged upon later in the communication. The physiological and pharmacological experiments which are reported are, it is felt by the writer, sufficient evidence to establish proof of the presence in extracts of all the tissues examined, with the possible exception of the prostate gland, of a common pressor principle. There are still a number of tissues to be submitted to study, so our

conclusions must be confined to those tissues which have been used. As the results have been so consistent, it will probably be found to be the case, that any tissue will yield a potent pressor extract of similar properties to those that have been the subject of this study.

One feature which makes a comparison of different tissues difficult is the variation in the amount of depressor substances present. Purification of the pressor principle appears to become increasingly difficult with increasing amount of depressors. As great wastage of the pressor principle probably occurs in purification, comparison of different tissues in relation to yield of the pressor principle is therefore of little value at this stage of the investigation. The liver, for example, is so rich in depressor substances, that it is very difficult to obtain a pure pressor effect with a liver extract, whereas in the case of the testis or certain commercial preparations of pancreatin the removal of almost all depressor substance may be readily accomplished.

One of the simplest methods of quickly preparing a potent pressor extract is to use a satisfactory brand of pancreatin. This should first be taken in solution in a small amount of water with the aid of heat. Five volumes of acetone are then added. The resultant precipitate is removed and the clear filtrate concentrated in vacuo to remove all traces of acetone. The concentrate is filtered and may be used at once.

Certain commercial preparations of pancreatin and pepsin are unsatisfactory for this purpose. This is no doubt due to the type of procedure used in their manufacture. Satisfactory pressor extracts have been obtained in the above manner from pancreatin (Armour, Wilson and Fairchild) and pepsin (Wyeth, Merck and Wilson).

This fairly simple extract, if made from commercial pepsin, is richer, as a rule, in depressor substances than if made from pancreatin.

Physiological studies with the pressor extract.

The study of the effect of the injection of the pressor extracts has been confined in this laboratory to animals under amytal anæsthesia. Rabbits, cats and dogs have been used with similar results in each instance. Prof. A. J. Carlson has very kindly check-tested a number of extracts on dogs under ether anæsthesia, and Prof. J. J. R. Macleod has likewise injected similar extracts into decerebrate cats. In all cases the results have been strictly comparable. Recently Dr H. H. Dale has very courteously sent me similar confirmatory results together with others which will be referred to later.

The blood-pressure response to injections of extracts from a number

of different sources is shown in Fig. 1. It should be noted here that the

Fig. 1. Dog ♂ 9·5 k. 1. Intravenous injection of 0·5 c.c. purified extract of ox spleen (300 gm.). 2. Intravenous injection of 1 c.c. extract of beef-steak (150 gm.). 3. Intravenous injection of 2 c.c. calf thymus gland (125 gm.). 4. Intravenous injection of 0·5 c.c. pig intestine (75 gm.). 5. Dog 87 ♀ 7·5 k. Intravenous injection of 5 c.c. extract of pig ovaries (100 gm.).

amount of the original tissue used is relatively very large. A considerable loss of the pressor component has undoubtedly occurred in the process of purification.

The mixed effect of an impure extract of testes is shown in Fig. 2. The extract used in this instance had not yet been fractionated with acetone.

The pressor effects of acetone extracts of commercial pepsin and pancreatin are shown to advantage in Fig. 3.

The injection of a simple dilute acetic acid extract made by boiling, filtering, and subsequent neutralisation before injection gives, as a rule, a pure depressor effect, or else a marked fall followed by a secondary rise in blood-pressure. It must be concluded that the presence of the pressor component is in such instances more or less masked by the more concentrated depressors. Fig. 4 is

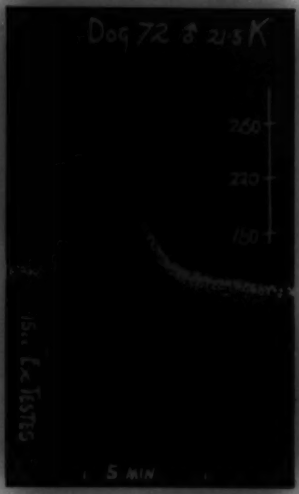

Fig. 2. Dog ♂ 21·5 k. 15 c.c. unpurified testicular extract injected intravenously. Note the mixed effect.

illustrative of this phenomenon. The injection of a simple extract of pig's stomach in dog 86 gave a profound fall in blood-pressure with no secondary rise. The same extract treated by the acetone method, as

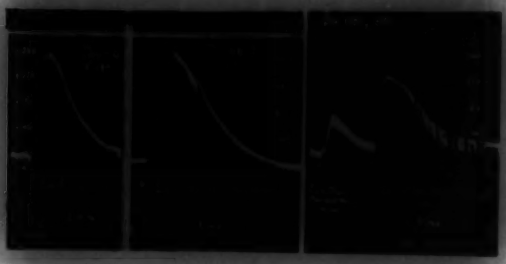

Fig. 3. Showing effect of intravenous injection of extracts made from commercial pepsin and pancreatin as indicated in the chart.

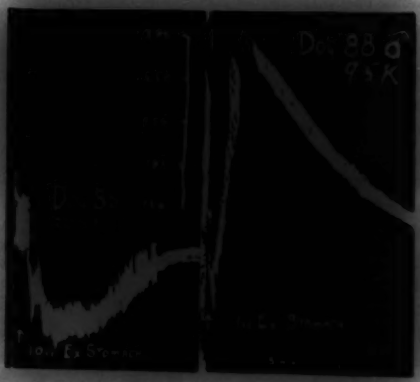

Fig. 4. Dog 86 ♂ 22·5 k. Effect of intravenous injection of 10 c.c. of a dilute acetic acid extract derived from 20 gm. of pig's stomach. Dog 88 ♂ 9·5 k. Same extract purified further by the acetone process. 200 gm. of stomach tissue used in second instance.

outlined above, gave in dog 88 a mixed effect with the pressor response predominating.

It is of interest to note that the pressor principle may be effective

when injected intramuscularly or subcutaneously. This is shown in Figs. 5 and 6.

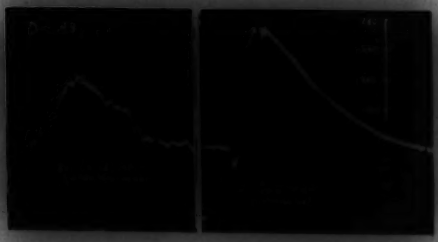

Fig. 5. Dog 89 ♀ 12·5 k. 1. Illustrating pressor response following intramuscular injection of 2 c.c. of extract of stomach. 2. Effect of a similar dose given intravenously.

Fig. 6. Dog 90 ♂ 19 k. 1. Pressor response following subcutaneous injection of extract of spleen. 2. Intravenous injection of 0·2 c.c. of same extract. 3. 0·1 c.c. and 0·05 c.c. respectively. 4. Illustrating antagonism of cocaine hydrochloride and pressor principle of extracts of spleen and beef-steak.

The degree of pressor response and also the duration of the effect of the purified pressor principle were found to be very intimately related to

the size of the dose administered. When the dosage is adjusted to produce a pressor effect of from 20 to 100 mm. of mercury, the relationship between effects may be practically quantitative (see Fig. 7).

Fig. 7. Dog 49 ♂ 24 k. Showing relationship of pressor response to size of dose of pressor principle administered.

No evidence whatsoever of reversal of effect has been obtained with the purified extracts. Injections have been repeated as many as thirty times without any alteration in the characteristic pressor response. In this respect this non-specific principle stands in sharp contrast to the pressor principle of the posterior lobe of the pituitary body.

A striking difference between the principle and epinephrine was found in the modification of the response of each as effected by cocaine. Cocaine hydrochloride administered intravenously had an antagonistic action upon the non-specific pressor principle. This antagonism may be manifested by a simple decrease in the pressor response of a definite dose or it may be extended to complete removal of the pressor effect, and even

Fig. 8. Dog 77 ♂ 22 k. Showing antagonism of cocaine hydrochloride upon pressor response of an extract of commercial pancreatin. Note gradual recovery of the pressor effect, also the complete neutralisation of the same when cocaine was administered a second time simultaneously with 0·2 c.c. of extract.

in certain instances to a reversal or a mixed effect. In no instance has synergy between cocaine and the principle been observed. It is of further interest in this connection that the antagonistic influence of cocaine is short lived. In the course of a few minutes it is reduced considerably, and in the course of one to two hours is completely lost. A further injection of cocaine however has, under such circumstances, again reduced or completely antagonised the effect of the pressor principle. The antagonistic action of cocaine hydrochloride is illustrated in Figs. 6, 8 and 9.

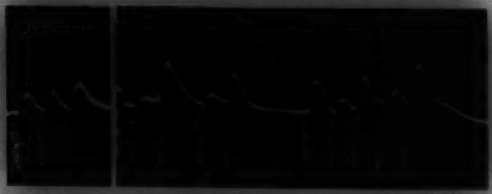

Fig. 9. Dog 82 ♂ 11·5 k. The antagonism of the pressor principle in a testicular extract by cocaine hydrochloride is here shown together with the non-antagonistic effect of the same upon the pressor principle of a prostatic extract.

It is of interest to note here that Tainter and Chang(7) have found an antagonistic action of cocaine upon tyramine, and that de Eds(8) has reported a similar result in the case of ephedrine.

Pharmacological evidence of the existence in the prostatic extract of a different type of pressor principle.

The pressor principle in extracts of a variety of tissues made by the acetone process has been shown to be antagonised by the intravenous injection of cocaine made either together with or just prior to the injection of the active extracts, with one exception, namely, the pressor principle predominating in extracts of the prostate gland of the bull.

Fig. 9 is illustrative of this type of experiment. Here the pressor effect of a potent testicular extract was very definitely antagonised by cocaine hydrochloride, whereas the effect of prostatic pressor principle was actually enhanced. This result has been repeated, and it would appear therefore that the prostatic extract contains a predominating pressor principle different in character from the non-specific principle which is the chief subject of this study. In regard to the action thereon of cocaine

the pressor action of the prostatic extract resembles that of epinephrine very closely.

The prostatic extract is peculiar in other ways. Thus whereas injection of a large dose of the non-specific pressor extracts results in a tremendous pressor response (pressures up to 350 mm. mercury), injection of large doses of the prostatic extract has not resulted in pressor responses of such magnitude. In one experiment, for example, the injection of a prostatic extract in doses graded from 0·125 c.c. to 1 c.c. caused a pressor effect of from 22 mm. to 105 mm. Hg. 4 c.c. of the same extract produced practically the same result as 1 c.c.

A detailed study of the pressor extracts derived from the prostate gland has not been made. It is emphasised however at this time that the prostatic extract is apparently different in character (as regards the chief pressor component) from other extracts made in a similar manner from the tissues which have been thus far examined.

Effect of other drugs.

The pharmacology of the non-specific pressor principle is a matter of considerable interest. The investigation has not been carried far enough as yet to permit of the establishment of the exact pharmacology of the principle. It differs in many essential points from both pituitrin and epinephrine. Atropine is without effect on the pressor response. Nicotine in doses sufficient to remove all appreciable pressor effect from subsequent doses has been noted to produce slight enhancement of the pressor effect of a definite dose. Gynergen (Sandoz) ergotamine tartrate (Stoll), administered intravenously to dogs in doses of 0·5 to 1·5 mg., has produced marked enhancement of the effect of a definite dose of the pressor extracts. Epinephrine was still effective however after such treatment, and the nerve endings of the vaso constrictors were therefore not paralysed. Dr H. H. Dale has recently informed me in a personal communication, which is quoted with his permission, that he has obtained reversal of the pressor effect in spinal cats which had previously been injected with sufficient ergotoxine to produce a complete reversal of the pressor action of epinephrine.

Non-effect on blood sugar concentration.

Intravenous injections of relatively large doses of the purified pressor extracts are well tolerated by normal rabbits. The blood sugar has been determined at frequent intervals in such experiments and has been found to remain within normal limits.

Effects on isolated organs.

The virgin uterus of the cat and rat and the gravid uterus of the rat were not appreciably affected by the addition of small amounts of purified pressor extracts to the Tyrode bath. Large doses had a depressant action. Isolated intestine was unaffected by small dosage, while large doses were depressant as in the case of the uterine strips. Similar results were obtained with the isolated cat's heart.

Characteristic features of the blood-pressure tracings.

The blood-pressure tracings indicate that following a latent period of a few seconds subsequent to the intravenous injection of a pressor extract, the rise in pressure occurs quite sharply, a peak point is reached, and then an orderly return to normal level follows. The duration of the level of increased pressure in relation to the intensity of the rise is much greater than in the case of epinephrine.

Occasionally a secondary rise may be manifested on the falling blood-pressure curve. This is an exception however to the general result.

When a massive dose has been administered phases of cardiac inhibition have been obtained much like those manifested in the case of the epinephrine injections. This also is an exception to the general rule.

Membrane manometer tracings of the blood-pressure, following intravenous injection of the pressor principle, are of diverse character. Coincident with the increase in blood-pressure and more or less paralleling it there is an increase in pulse pressure. The heart rate may be accelerated or again it may show little change.

General discussion.

The presence of pressor substances in extracts of various tissues, apart from the well-known pressor constituents of the extracts of the posterior pituitary lobe and of chromaffin tissue, has long been known. Whether the pressor effects which have been observed by others have been due to the non-specific principle which has been described herein is not determined. It is a possibility that, in the method of extraction which has been used, something occurs which gives rise to the pressor component. This the writer considers very improbable. Earlier workers have emphasised the destructive effect of heat upon the pressor component in their extracts. The non-specific pressor substance with which this paper deals appears to be unaffected by heat *per se*.

Also one cannot be positive that one is dealing in each case with the

same chemical individual. Efforts to obtain the pressor constituent in crystalline form have so far failed, and one can therefore only rely on physiological and pharmacological tests. Such tests would indicate that, in the case of the extracts examined, all contain a similar component, with the exception of the prostate extract, which appears to contain a pressor substance having a somewhat different pharmacology.

The physiological data which have thus far been obtained relative to the action of the pressor component tend to point to a substance having a very selective stimulating effect upon certain peripheral structures and in the main confined to the circulatory system. In some respects it resembles epinephrine, while in others it is more like tyramine and ephedrine. The antagonism of the pressor response by mild systemic cocainisation is diametrically opposed to the behaviour of epinephrine under like circumstances. It is however quite in line with the behaviour of tyramine and ephedrine. The antagonism by cocaine of the pressor response to both of these latter drugs has been established quite recently by Tainter and Chang(7), and de Eds(8) respectively. The former authors concluded from the results of perfusion experiments with isolated organs that cocaine modifies the responses of vessels to tyramine and epinephrine in an opposite manner and through different mechanisms. They held that it diminished or abolished the tyramine pressor effect through some depression of the muscle substance and that it increased the epinephrine pressor response through some sensitisation of the sympathetic nerve endings.

The enhancement of the pressor response of the non-specific pressor substance by small doses of ergotamine (Stoll) obtained so frequently by the writer is of itself difficult of interpretation. If one is correct in assuming that the action of this drug, even in small doses, is to cause depression of the nerve endings of the sympathetic system, then enhancement of the pressor response of the non-specific principle following the administration of ergotamine could not be taken as evidence for the selective stimulation of the vaso-constrictor nerve endings. Dr Dale's results, previously referred to, help to clear up this difficulty. He obtained reversal of the pressor response when his spinal preparation had received sufficient ergotoxine to completely abolish the pressor action of epinephrine. Tyramine in this particular experiment still gave a pressor response, according to Dr Dale's statement.

It may be concluded therefore that the action of small doses of ergotamine sensitises some structure or structures upon which the pressor principle acts, whereas doses which paralyse the vaso-constrictor

nerve endings effectually prevent the pressor component from exercising its characteristic action. As the pressor response is not decreased by nicotine in doses sufficient to remove the pressor action of nicotine itself, it may be provisionally concluded that the non-specific pressor principle acts on sympathetic nerve structures somewhere between the terminal ganglia and the end plates. It would harmonise with the cocaine antagonism experiments if a specific circulatory muscle stimulatory action be ascribed also to the principle.

Assuming that the non-specific pressor principle is not an artifact but actually exists preformed in the tissues, one is led to ask the question as to what is its significance in normal physiological economy. Does it suggest that tissues contain within themselves some sort of autocoid capable of effecting a local regulating action upon the rate of blood flow through them: or may it be possible for one tissue, under certain conditions of activity or stimulation, to release such an autocoid into the general circulation? If either of these suppositions should be true this work would lend support to the recent findings of Vincent and Thompson(6) referred to earlier in this paper.

Summary.

1. A method has been described for the production of extracts containing a powerful pressor component.

2. A wide variety of tissues have been used as a source of this non-specific pressor substance.

3. The pressor extract derived from the prostate gland has been found to be essentially different in character from pressor extracts obtained from other tissues studied.

4. The non-specific pressor substance has a very interesting pharmacology.

(*a*) It does not effect blood sugar.

(*b*) The pressor response is enhanced by small doses of ergotamine. A large dose may cause reversal of the pressor action (Dale).

(*c*) The pressor effect is antagonised by the intravenous injection of small amounts of cocaine hydrochloride.

(*d*) Atropine does not affect its action.

(*e*) Nicotine does not diminish the pressor effect but may cause enhancement.

(*f*) Small amounts have little or no effect upon isolated intestine, uterus or heart. Large doses are depressant.

(*g*) The pressor response (both height and duration) is directly related to the size of dose within certain limits.

(*h*) Repeated injections at short intervals do not alter the character of the pressor response.

5. Whether the active principle exists as such in the tissue, or is produced during the process of making the extracts, is not established.

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PLURISEGMENTAL INNERVATION IN THE FROG.

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During the past few years there has been a renewed interest in the arrangement of the nerve fibres supplying striated muscle. In 1924 Dr P. W. Stiles and the author(5) published evidence indicating that in the gastrocnemius muscle of the frog a certain proportion of the fibres received nerve elements from more than one segment of the spinal cord. Shortly thereafter there appeared the results of two independent investigations on the same subject. One of these, Beritoff(1, 2), supported our conclusions, while the other, Samojloff(16), presented evidence from which it was concluded that the two nerve roots concerned innervate separate muscle fibres. Since this time many investigators have studied the problem without coming to a general agreement; the consensus of opinion, however, seems not to favour the existence of a plurisegmental innervation of the individual fibre in the frog.

Since the mode of innervation has an important bearing on the evidence relating to the question of local or general fatigue of the muscle fibre (see Cattell and Stiles(6) and Beritoff(2)), it has been thought worth while to secure additional evidence, and to make a survey of the literature with the purpose of determining to what extent the opposed views may be reconciled.

It may be stated at the outset that the evidence of a plurisegmental innervation in the frog is strongly supported by the experimental evidence, and that practically all the arguments which have been advanced opposing this conception lose their force when consideration is given to the fact, upon which there is general agreement, that not more than half the fibres of a muscle are doubly innervated. Another factor which has added to the confusion is the great individual variation which obtains in the frog.

Method. Further data bearing on the distribution of the nerve fibres from separate spinal roots have been secured in two ways: experiments have been carried out in which there has been made a comparison of the tensions developed by the sartorius muscle of the frog when the roots innervating it are stimulated singly and together, and, secondly, a

study has been made, in an isotonically arranged gastrocnemius muscle, of the degree of summation resulting when two successive stimuli reach the muscle through different nerve pathways.

The tension records from the sartorius were obtained with the muscle *in situ*, the tendon merely being separated at the knee joint and attached through a rigid connection to an isometric lever. The muscle was also partially freed of its connective tissue attachments to neighbouring muscles, care being taken not to injure its nerve supply. The position of the origin of the muscle was fixed as securely as possible by means of pins passed through the pelvic skeleton and the knee joint into a board. The isometric lever employed was the torsion wire myograph made in accordance with the specifications given by Fulton (10). Two methods were used to register the tensions developed: (1) by the attachment of a light straw to the lever ordinary kymograph tracings were made on smoked paper; and (2) by the use of a small mirror placed at the axis of rotation of the free end of the wire a beam of light was reflected into a camera and recorded on a moving strip of bromide paper.

The nerve roots were stimulated through a double set of shielded electrodes mounted in a small block of hard rubber which was securely fastened within the abdominal cavity. Each of the two roots (VII and VIII) supplying the sartorius muscle was cut close to its emergence from the vertebral column and each was placed across one pair of electrodes. A short tetanic stimulus, above maximum in intensity, was applied successively to each of the two roots separately and together. In order to control a possible modification of tension due to fatigue the stimuli were always repeated in the reverse order.

Should there be spread of the stimulus from one root to the neighbouring one, very misleading records would result, and Fulton (11) has suggested such an error as a possible explanation of the excess tensions occurring when the roots are separately stimulated, over that recorded for the simultaneous stimulation of the same roots. In the present experiments special precautions were taken to rule out this possibility, and every preparation was controlled by the following procedures: the root giving the smallest tension was stimulated with shocks of increasing intensity until an increase in the tension developed by the muscle resulted, thus giving evidence of a spread to the other root. In every case the experiments were carried out with stimuli of an intensity well below that found to affect the adjacent root. After completing the observations, the second control test was carried out as follows: the muscle was stimulated to complete fatigue through one root, following which the other root was stimulated, and if there had been no spread of current a second fatigue curve similar in all respects to the first was obtained (Cattell and Stiles (6)). Actually with a properly arranged preparation there is a wide margin of safety, represented by the intensity of the stimulus giving a maximum response in the muscle and that spreading to the neighbouring root. With the apparatus used (large Stoelting induction coil with a single dry cell) a maximum current was usually obtained with a secondary coil at an angle of 45° and fully extended (210 mm.), whereas spread did not occur until the coil was parallel to the primary at a position of 130 to 150 mm.

For the observations on the degree of summation resulting when successive stimuli reach the muscle through different nerve roots the isolated gastrocnemius muscle was used, and also, in a few cases, the sartorius muscle *in situ*. In the earlier experiments the muscle was attached to an ordinary Harvard isotonic muscle lever and the degree of shortening registered either on smoked paper or, through an optical system, on bromide paper. The technic was later improved by the substitution of a light torsion wire lever to the axis of which a small mirror was attached. In order to increase the range of movement of the muscle without greatly influencing the tension, a small coiled spring was interposed between the muscle and the lever in many experiments. Such a system, while not strictly isotonic, allows full freedom of movement, and it has the advantage over the ordinary

isotonic lever that error due to the inertia of the system is practically eliminated. Separate induction coils were used for the stimulation of each root, and in carrying out an experiment each root was first stimulated in turn separately, then both were stimulated together, and finally a stimulus was sent successively to each root so timed by means of a Lucas pendulum that they produced summation in the muscle.

These experiments were based on the following reasoning: when two successive maximum stimuli are so timed as to produce summation of muscular contraction, the greater shortening must be due to a greater response of the individual fibres. On the other hand, if the two stimuli affect separate fibres only, the degree of shortening cannot be appreciably greater than that resulting from the simultaneous stimulation of both roots. If a greater shortening occurs following the successive application of the stimulus to the two roots than it does from the simultaneous stimulation of these same roots, we have definite evidence that *some* of the muscle fibres are plurisegmentally innervated.

Results. The tension figures obtained from the sartorius muscle in nineteen experiments are summarised in Table I. In addition there were

TABLE I. The tensions developed by the sartorius muscle in response to successive and simultaneous stimulation of the VIIth and VIIIth roots.

Experiment	Root VII grm.	Root VIII grm.	Sum roots VII and VIII grm.	VII and VIII stimulated together grm.	Superiority of single root stimulation p.c.
1	33·1	99·0	132·1	111·6	18·4
2	38·9	54·0	92·9	64·8	43·3
3	90·1	97·7	187·8	108·1	73·8
4	28·9	111·8	160·7	113·1	24·5
5	80·6	124·7	205·3	130·1	57·9
6	33·1	118·4	151·5	121·0	25·2
7	16·4	108·4	124·8	108·7	14·8
8	44·4	51·7	96·1	75·6	27·0
9	23·6	89·1	112·7	90·7	24·3
10	54·0	61·9	115·9	78·8	47·0
11	45·0	81·7	126·7	88·9	42·5
12	18·0	103·7	121·7	108·4	12·3
13	15·1	83·2	98·3	83·8	17·2
14	12·0	90·1	102·1	90·1	13·3
15	27·4	69·1	96·5	72·0	34·0
16	39·4	89·8	129·2	96·4	33·9
17	58·3	110·2	168·5	122·2	37·9
18	58·1	89·3	147·4	102·2	44·2
19	43·6	85·0	128·6	106·7	21·0

four experiments in which stimulation of the VIIth root resulted in no response of the muscle, the entire innervation taking place through fibres in the VIIIth root. The excess tension of the sum of the responses to separate stimulation of the two roots over that for their simultaneous stimulation, listed in the last column of the table, varied all the way from 12 p.c. to a maximum of over 73 p.c., and averaging 32·2 p.c. The extent of overlapping, i.e. of plurisegmentally innervated fibres, is conditioned in the case of this muscle to a large extent by the number of fibres which reach it through the VIIth root. Where no fibres are present in this nerve there can, of course, be no overlapping, but in some of the cases included

in the table a relatively small effect results from stimulation of this root so that the number of fibres doubly innervated is necessarily limited. However, where the effect of the VIIth root is slight the tension developed on stimulation of the VIIIth root is hardly inferior to that produced when both are stimulated together, indicating that practically all of the meagre supply of motor axons in the VIIth root go to muscle fibres which are also supplied by the VIIIth root. If, on the other hand, we select only those frogs in which 50 p.c. or more of the total power of the muscle may be commanded through the VIIth root, we get evidence of a very much greater overlapping, averaging 46 p.c. for the seven examples included in the table.

The second group of experiments in which evidence on the question of double innervation was sought by the influence on the contraction of the gastrocnemius muscle of two stimuli applied successively to the VIIIth and IXth roots, and so spaced that summation of contraction resulted when they were applied to the same nerve, gave clear-cut results. Invariably the shortening of the muscle in response to successive stimulation of the two roots was considerably greater than that resulting from their simultaneous stimulation. Typical examples are reproduced in Figs. 1 and 2. Fig. 1 shows summation in a preparation in which the

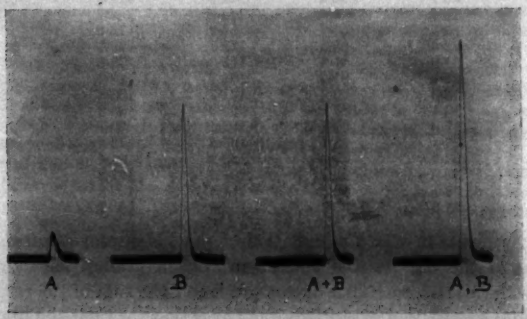

Fig. 1. Photographs showing summation in the response of the gastrocnemius muscle when stimuli are sent successively to each of the two nerve roots supplying the muscle. *A*, VIIIth root. *B*, IXth root. *A* + *B*, both roots simultaneously. *A*, *B*, both roots successively.

nerve distribution to the muscle through the two roots was very unequal, while Fig. 2 shows a similar result in another preparation in which each

of the two roots produced approximately the same degree of shortening in the muscle. In thirteen experiments the average shortening of the

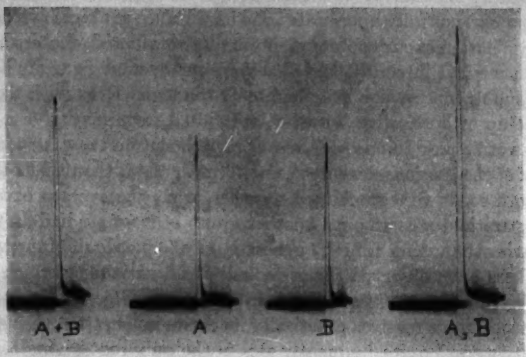

Fig. 2. Same as Fig. 1, but taken from a preparation in which each root produced approximately the same degree of shortening in the muscle.

muscle was 41 p.c. greater when the two roots were stimulated successively at an interval of 0·012 sec., than they were when the same nerves were stimulated simultaneously. Since in the latter case all of the fibres of the muscle innervated by the two roots would be stimulated, a further shortening on introducing an interval between the stimuli applied to the two roots indicates a wave summation, and it follows that at least a few fibres must have received stimuli from the two approaches, *i.e.* they are plurisegmentally innervated.

Discussion.

Evidence from tension. A number of recent investigations have been concerned with the relative tensions obtained as a result of stimulation of the gastrocnemius through one or both roots. Without exception the sum of the tensions from separate root stimulation has been found to be greater than that resulting from their simultaneous stimulation, although the actual figures vary greatly. Fulton(11) obtained the lowest result; an overlapping of only 11 p.c., representing the approximate figure for a number of experiments, while our average figure of 70 p.c. from thirteen experiments is the highest(6). Fulton believes that the small superiority of separate root stimulation observed by him is to be explained by the

slightly greater shortening of the muscle fibres which results when all are active at the same time, under which conditions less tension is developed by the individual fibres. Before the appearance of Fulton's paper we had not fully appreciated the importance of a rigid system, and a re-examination of our apparatus shows that we allowed greater shortening than we had intended, and therefore our average figure indicating 70 p.c. double innervation is undoubtedly too high. Even with the most rigid set-up of apparatus, however, individual frogs may give a figure in excess of 70 p.c. In one such recent experiment on the gastrocnemius the sum of the tensions developed was 76 p.c. greater than that obtained when both roots were stimulated together, while the average excess for five preparations was 48 p.c. Another group of frogs studied at Woods Hole during the summer of 1927 indicated only 38 p.c. double innervation. All workers have observed the great individual variability which exists in the distribution of the spinal nerves of the frog. Rarely, for example, the gastrocnemius receives practically its entire innervation through the VIIIth root, and quite frequently this is the only root giving fibres to the sartorius muscle. Whether, in these frogs, this indicates a unisegmental innervation or whether fibres from more than one level of the cord leave in a single root is a question which must be decided by anatomical studies. The instances cited serve to emphasise the great variability of the material and suggest the explanation for the wide divergence in the results reported from different countries by various observers. On account of this variability it is probably better to consider the nerve distribution of individual frogs rather than to deal with averages.

Other figures include those of Samojloff and Wassiljewa(17) indicating an overlapping of 20 p.c., Boyd(3) with an average figure of 23 p.c. in thirty-four experiments, and Katz(15) who gives 41 p.c. as the average figure in sixteen experiments, with variations ranging between 17 and 77 p.c. Similar observations have been reported by de Boer(9) without giving numerical data. Beritoff(2) found that stimulation of either root singly gives rise to only 15 to 20 p.c. less tension than when stimulated together, indicating an overlapping in the innervation of between 60 and 70 p.c. However, according to Fulton(12), p. 183, his method of recording permitted shortening.

The existence of a plurisegmental innervation in certain skeletal fibres of the frog can hardly be questioned from these results. The contention of Katz(15) that, in the case of the gastrocnemius, tension gives a misleading picture of the number of fibres in action because they pull

to a certain extent against each other is discussed later. However, in the case of the experiments described in the present paper with the parallel fibred sartorius muscle such a complication does not exist, and still the results of tension measurements give a clear indication of a considerable number of plurisegmentally innervated fibres. Our average figure of 32 p.c. may possibly be a trifle high, because of the slightly greater shortening permitted in the individual fibre when all are contracting together, as compared to the conditions when a somewhat smaller number are active through stimulation of a single root. In the case of the gastrocnemius, Fulton(12), p. 120, has reported four experiments in which the average tension developed by the muscle was decreased by nearly 30 p.c. when the amount of shortening permitted was changed from 0·02 mm. to 0·52 mm. A similar allowance applied to the difference in shortening in the present experiments would account for but an insignificant part of the excess recorded for the separate stimulation of the roots. On account of the great variability it is of greater interest to consider the conditions as they exist in individual muscles. In certain instances, if we can use the results of root stimulation as our criterion, none of the fibres composing the gastrocnemius and sartorius muscles receives nerve elements from more than one level of the cord, but, on the other hand, a considerable proportion of these muscles show a superiority of 50 p.c. or over in the sum of the tensions developed on separate stimulation of the roots as compared to the tension resulting from their simultaneous stimulation. In these muscles there can be no question but what many fibres are bi-segmentally innervated.

Electrical evidence. The action current has been employed by Samojloff(16), Samojloff and Wassiljewa(17), Fulton(11), and de Boer(9) in relation to the question of plurisegmental innervation of the gastrocnemius, on the assumption that the size of the excursion bears a direct relationship to the number of active fibres. The experiments of Watts(20) and others establish such a relationship for parallel fibred muscles, but unfortunately complicating factors enter into the conditions necessary in studying the influence of the two roots. There is, however, general agreement that the sum of the action currents obtained by separate stimulation of the two roots of the gastrocnemius approximates that obtained by their simultaneous stimulation, and these facts are cited as proof of the absence of plurisegmental innervation. Fulton reports superiority of only from 3 to 10 p.c. in the summed effects from the separate roots. Samojloff's records, taken from the gastrocnemius of the frog, show that the size of the excursion varies with the position of

the leading-off electrodes, and that under certain circumstances the deflection may be reversed in direction, thus demonstrating that the size of the excursion depends on factors other than the number of active fibres. This result becomes clear when we take into account the structure of the gastrocnemius muscle, *i.e.* a large number of short fibres of diverse orientation, and also the fact that all records have to a greater or less degree a diphasic character. The writer believes that in view of these conditions we are not justified in using the electrical evidence as a proof that no fibres are plurisegmentally innervated; it merely indicates that some fibres do not receive the double nerve supply. Moreover, we have no knowledge of the effect of a simultaneous stimulation of the fibre from two approaches on the magnitude of the action current: it is not unlikely that a greater deflection would result, either because of two foci of activity, or through the promotion of a more rapid reaction, thus accounting for the relatively great effect of double root stimulation, as compared to the results of tension records. Samojloff's finding(16) that the action current shows certain characteristic effects as the result of a second stimulus applied during the refractory period of the first, but through a different root, is doubtless to be explained by the fact, upon which there is general agreement, that only part of the muscle fibres would be brought into action by the first stimulus.

Evidence from heat production. Katz(15) has measured the heat production of the gastrocnemius muscle of the frog following single and combined stimulation of its two nerve roots, and his data represent the most important evidence that has thus far been adduced *against* the conception of plurisegmental innervation of individual fibres. In a series of sixteen experiments the sum of the heats obtained on separate stimulation of the two roots was on the average only 1 p.c. greater than the heat obtained by stimulating both components together, the extremes of variation being $\pm$ 13 p.c. In the same preparations the measurement of tension gave an average superiority of 41 p.c. for the separate stimulation of the roots. Katz suggests that this discrepancy might be explained through a consideration of the various directions in which the fibres exert their force, the tensions being the resultant and not the sum of the individual tensions of the fibres. It is also assumed that when one root alone is stimulated the contracting fibres turn more or less parallel to the long axis of the muscle, whereas when both sections of the muscle contract together they pull, to a certain extent, against each other. Heat, on the other hand, has no directional quality and should give a true measure of the number of active fibres.

In view of the fact that Katz's investigations oppose a considerable body of evidence indicating that a certain proportion of muscle fibres *are* doubly innervated, a confirmation of his results is greatly to be desired. They lead to the curious conclusion, pointed out by Stiles(19), that the efficiency of the muscle becomes less when the entire mass of tissue is at work than when a fraction of it is idle. In one of his earlier papers Hill(14) has pointed out the difficulty of making accurate measurements of the heat production in large masses of muscle tissue such as obtains in the case of the frog's gastrocnemius. It was observed, for example, in certain instances in the gastrocnemius that if fibres close to the junction of the thermopile contracted, but not elsewhere, the heat was conducted away so rapidly that no delayed heat production could be demonstrated. Some observations of Hartree and Hill(13) suggest that under certain conditions where only a fraction of the muscle is active the heat reading is too low. They found in the sartorius muscle that as the strength of the stimulus is increased from the threshold to the maximum value the ratio of heat to tension is increased on the average 26 p.c. The increase in this ratio is accounted for by the assumption that a change has occurred in the manner of response of the individual fibre to the increasing stimulus, necessitating a modification of our usual conception of the all or none law. To the present writer, however, it seems more likely that when only a few fibres are active in a muscle mass the heat liberated is less completely recorded than when all the fibres take part. If such is the case we have an explanation of the discrepancy between the recorded heat production and tension in the experiments of Katz, *i.e.* it is possible that a greater proportion of the heat produced is recovered when all the fibres are active through stimulation of both roots, than when only about 70 p.c. of the fibres are active through stimulation of one root only. This is made more probable by the fact that the thermo-junctions were arranged in a circle around the belly of the muscle, and that many fibres are less than 10 mm. in length, and therefore do not extend the full length of the muscle. Experiments are now being made to test this suggestion.

Evidence of a qualitative nature. Qualitative evidence bearing on the question of plurisegmental innervation in the frog has been adduced by a number of workers through various ingenious procedures. The results in general have been accepted by their authors as evidence that no fibres are doubly innervated, whereas, in my opinion, there is only justification for concluding from these experiments that *some* fibres are unisegmentally innervated. Those who have taken the viewpoint

that muscle fibres receive nerve elements from only one source have failed to take into account the fact that the opposing side has never claimed that the evidence indicates a double innervation of more than a certain proportion of the fibres of a given muscle, and this has led to much confusion. Take for example the striking difference in the shape of the contracted muscle resulting from stimulation of the VIIIth root as compared to the IXth, observed by so many workers including de Boer(8) and Boyd(3) with the silhouette method, and Samojloff and Wassiljewa(18) by means of a stylus passed through the middle of the muscle. This variation in the activity of different parts of the same muscle is quite in accordance with the data here reported; no complete overlap in the distribution of the nerve fibres from the two roots being indicated.

Similarly the results reported by de Boer(7) on the effects of veratrine do not argue against the existence of plurisegmental innervation. De Boer found, and I have repeated his experiments with exactly similar results, that the veratrine effect is abolished only for the particular root stimulated, the other approach giving the typical curve. This result is to be expected on the ground that about half the fibres are unisegmentally innervated; the fibres which escape stimulation from the first root being sufficient to give the typical curve, although in isometric records to a lesser degree.

As further evidence against the existence of a plurisegmental innervation Samojloff and Wassiljewa(17) report that stimuli applied through one root at the rate of 5 per sec. resulted in a rhythmic notching in a tracing being recorded of a fused tetanus from stimulation of the other root. Of this type of experiment Fulton(12), p. 185, makes the comment that "one could scarcely ask for a more convincing demonstration of monosegmental innervation." It is clear, however, that such experiments merely prove the unisegmental innervation of some fibres in the muscle.

The experiments reported in the present paper showing a wave summation in the response of the muscle to a stimulus applied to one nerve root following shortly after a stimulation of the other root, definitely prove that *some* fibres are doubly innervated. Since the muscle was allowed to shorten this result gives but a qualitative answer and gives no information regarding the number of fibres so innervated. The degree of shortening registered by an isotonically contracting muscle varies but little through a rather wide range in the number of fibres participating, and therefore wave summation might be in evidence as the result of the double stimulation of relatively few muscle fibres. Summation may also

be demonstrated in an isometrically arranged preparation, but due to the fact that those fibres which are not doubly innervated are not simultaneously stimulated, the number of fibres participating in the summated response cannot be accurately judged by the tension developed. For an estimation of the number of plurisegmentally innervated fibres in a muscle, the sum of the tensions developed by individual root stimulation must be compared to the tension resulting from their simultaneous stimulation. Such experiments demonstrate a definite but variable proportion of plurisegmentally innervated fibres in the frog.

The conclusion here reached is strongly supported by the observation, Cattell(4), that frogs in which either the VIIIth or IXth root is transected on one side show practically normal function on the operated side after several months, and also by certain histological studies[1].

Summary.

1. Summation of contraction, resulting from the application of two successive stimuli to the isotonically arranged gastrocnemius muscle of the frog, still occurs when the two stimuli are applied in turn to the VIIIth and IXth spinal roots.

2. In the frog the paralleled fibred sartorius muscle is usually innervated by the VIIth and VIIIth spinal roots, and in these cases the sum of the tensions developed on separate stimulation of the two roots was greater than the tension developed when they were stimulated simultaneously, by an amount varying in nineteen experiments, between 12 and 73 p.c.

[1] Dr Adrian has kindly called my attention to two important papers by Beritoff which I had previously overlooked. In the first of these papers (*Zeit. f. Biol.* 85. p. 509, 1927) Beritoff showed that when the leading-off electrodes were situated at the nerve-free portion of the vastus externus or sartorius, the sum of the action currents from separate stimulation of the two roots was greater than that obtained from their simultaneous stimulation, whereas the total magnitude of the action currents in these two conditions approached equality when led off from the part of the muscle richest in nerve endings, thus indicating that the size of the action current bears a relationship to the number of endplates present in the muscle. There thus exists experimental evidence supporting the suggestion made in the present paper under the heading "electrical evidence." In the second paper (*Zeit. f. Biol.* 85. p. 521, 1927) Beritoff presents evidence indicating that previous activity of the vastus externus of the frog, induced by stimulation of one root, modifies in various ways the electrical and mechanical response of the muscle to stimulation of the other root. These changes include the production of a refractory period, modification of the curves in the direction of fatigue, and increased excitability under certain conditions. Thus the experiments described in the present paper are confirmatory of those of Beritoff in indicating the presence of plurisegmentally innervated muscle fibres in the frog.

3. The data reported confirm previous work indicating the existence of a varying proportion of plurisegmentally innervated fibres in the gastrocnemius and sartorius muscles of the frog.

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A METHOD OF OXYGENATING BLOOD.

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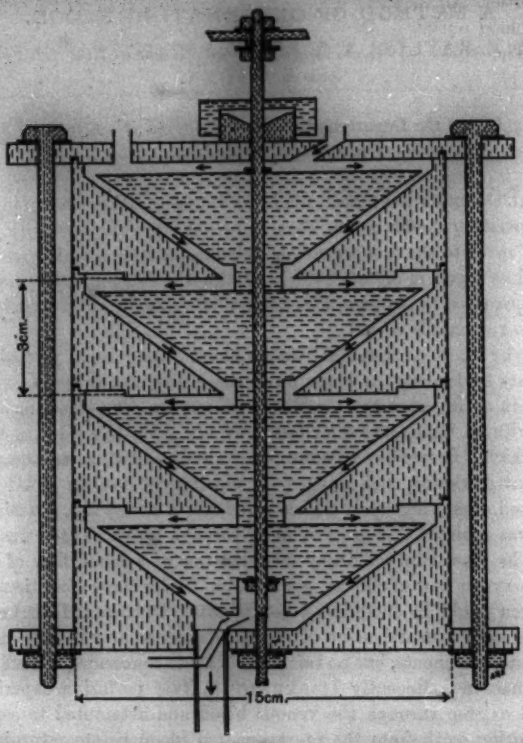
Several attempts have been made to obtain a satisfactory method of re-oxygenating venous blood. These, however, have only met with partial success owing to the ease with which blood froths when unduly agitated; and unless blood is agitated during artificial oxygenation a very large surface relative to volume is necessary in order to effect oxygenation within a reasonable period.

Hooker(1) re-oxygenated venous blood during brain perfusion experiments by leading the blood on to the centre of a rubber disk which rotated in an inverted bell jar filled with oxygen. The blood, as a result of centrifugal force, was distributed evenly upon the sides of the jar down which it flowed in a thin film. Richards and Drinker(2) supplied the isolated kidney with oxygen by passing the venous blood into a perforated glass ring upon which was hung a heavy silk curtain. The whole was enclosed in a glass cylinder through which oxygen was circulated. The maximum efficiency recorded was at a blood flow of 50 c.c. and an oxygen flow of 800 c.c. per minute. Under these conditions 3 c.c. of oxygen could be introduced per minute. Drinker, Drinker and Lund(3) used a modification of Hooker's oxygenator in bone marrow perfusion experiments, but no reference is made concerning the efficiency of the machine. Recently Issekutz(4) in liver perfusion experiments bubbled oxygen through the venous blood and attempted to separate the resulting froth from the re-oxygenated blood before returning the latter to the liver.

During the course of other investigations we desired an oxygenator capable of introducing at least 12 c.c. of oxygen per minute regardless of blood flow. It was also necessary that the amount of blood in the machine at any moment be at a minimum, and that the air volume be under a litre. Various principles were tried and rejected either because the oxygenator was disproportionately large or because of the formation

of froth. The type of oxygenator finally adopted is represented in the accompanying figure. A series of inverted truncated cones is held together on an axle and is capable of rotation through the agency of a



worm gear. Power is supplied by a small D.C. 4-volt motor resting on the top of the oxygenator and the speed of rotation controlled by a resistance. These movable cones are intercalated between a series of stationary plates which "nest" into one another accurately at their periphery; air-tight joints are effected by thin rubber washers. The top of the oxygenator is covered with a plate which bears a stuffing box through which the axle passes, and also a blood inlet pipe and an air inlet pipe. Attached

to the bottom plate is an adjusting device by means of which the position of the rotating plates may be adjusted relative to that of the stationary plates. To one side of this is a large circular aperture which serves as a blood outlet. Air is removed through a small pipe running up the centre of the blood outlet. An air trap is placed at the bottom of the blood outlet. The whole is held together by bolts running between the top and bottom plates.

Blood entering the machine falls on the first revolving plate and is thrown in a thin film to the periphery where it collects on the upright wall of the first stationary plate, flows down the inclined surface, and passes through the central orifice on to the centre of the second revolving plate where the process is repeated.

In order to obtain the maximum amount of surface for a given volume of air it was necessary to reduce the clearance between the revolving and stationary plates to an absolute minimum. By observation it was found that the film of blood became thickened in two regions, viz. at the periphery of the revolving plates and at the circumference of the orifice of the stationary plates. These thickened rings have a depth of 1 mm. or more at the speeds which the plates are rotated, whereas in other regions the film varies between 0·2 to 0·4 mm. in thickness, depending on the blood flow. Consequently by increasing the clearance where these rings occur (see diagram) it was possible to reduce the clearance to 2·5 mm. in other regions without danger of the blood film coming into contact with the opposing surface. If such interference does occur froth is formed.

The rate at which the plates are rotated is an important factor. Up to a point the greater the speed of rotation the thinner the film on the rotating plates and the less the amount of blood held in the machine at any moment. It might be inferred that a very thin film moving at a high speed would be the most effective in causing a high rate of oxygen intake, but the observations of Hartridge and Roughton(5) indicate that for films of the order of thickness of capillaries the rate of diffusion of oxygen is large compared to the velocity of reaction of hæmoglobin and oxygen, consequently it is possible for layers, say 50μ from the surface of the film, to receive oxygen before the upper layers are saturated. Moreover, if blood strikes the walls of the stationary plates at too high a speed froth is formed. By experiment it has been found that 150–200 revolutions per minute is the most satisfactory speed, and in all subsequent discussions it is implied that the plates are revolving at this speed.

In Table I the actual amount of oxygen taken up by blood passing

TABLE I.

Gas in oxygenator	Blood flow (c.c./min.)	P.c. saturation of blood		No. of plates	C.c. O_2 per min. per plate
		Incoming	Outgoing		
Air	150	21	77	8	1·8
"	60	21	100	8	1·0
"	150	—	—	2	0·8
"	150	67	72	2	0·75
"	80	38	73	4	1·1
"	90	3	15·5	2	0·9
"	120	3	49	5	1·7
"	70	3	62	5	1·3
"	106	23	61	5	1·4
5 p.c. CO_2 in air	200	54	77	5	1·6
"	200	77	89	5	1·0
"	200	89	97	5	0·5
Oxygen	80	42	97	4	1·7
"	125	2	42	2	3·9
"	210	33	54	2	4·0
"	210	55	91	5	2·7
"	260	3	72	5	5·4
"	202	24	80	5	4·0

through oxygenators containing different numbers of plates is given. Air, oxygen, and mixtures of air and carbon dioxide were passed into the machine as indicated. The blood flows varied between 60 and 260 c.c. per minute without appreciably influencing the rate of oxygen intake. The temperature at which the determinations were made was always above 25° C. From this table it is obvious that one rotating plate and one stationary plate are together capable of introducing 0·8 c.c. of oxygen per minute (regardless of blood flow) when air is being circulated through the machine and approximately 4·0 c.c. when the oxygenator is running on pure oxygen.

It is to be expected that the rate at which the blood in the oxygenator absorbs oxygen is roughly proportional to the difference in partial pressure of oxygen between the atmosphere and the incoming blood. We may take this latter (for about 40 p.c. saturation) to be approximately 2 p.c. of an atmosphere, so that the pressure head would be 98·0 p.c. of an atmosphere when pure oxygen is used and 19·0 p.c. of an atmosphere when air is used. The ratio between these pressure heads is 5, which is the ratio between the rate of oxygen uptake by the blood when the machine is running on oxygen and on air (4·0/0·8). This point is further exemplified by the data given when the oxygenator is run on the 5 p.c. CO_2 in air mixture. It can be seen from any dissociation curve of blood that the partial pressure of oxygen in equilibrium with blood in the presence of 5 p.c. CO_2 rises from 4 to 6 p.c. of an atmosphere at 60 p.c. saturation, and to

8 p.c. of an atmosphere at 90 p.c. saturation. This causes a very significant fall in the pressure head available for diffusion, and accordingly the rate of oxygen intake by the blood passing through the machine is reduced.

From the above data it can be seen that an oxygenator of this type containing five revolving plates will, when running on pure oxygen, introduce roughly 19·0 c.c. of oxygen per minute into the blood running through it. The air volume of such a machine is 600 c.c., and when running at a speed of 200 revolutions per minute contains 54 c.c. of blood at any given moment. It may therefore be used to supply, with completely arterial blood, any organ whose oxygen requirements are less than this amount. As has already been indicated it is necessary to circulate oxygen or air in the machine at a rate great enough to prevent the undue accumulation of CO_2 since this will decrease the available pressure head of oxygen.

The material from which the plates are made may be left to the discretion of the experimenter. We have used aluminium, although this requires careful cleaning after each experiment. In time the plates become covered with a coating of oxide, but this does not appear to have any toxic effect either upon a perfused heart or kidney. Indeed, from the experiments of Dollken(6) and Jalan de la Croix(7), there is no reason to expect that aluminium or aluminium oxide will have any toxic effect during an experiment lasting for a period of several hours. It is necessary, however, to select some substance which can be turned accurately upon a lathe. The slightest trace of grease upon the surface of the plates prevents the formation of a uniform film of blood. It is therefore advisable to avoid using soap in cleaning, and instead to dip the plates into a dilute solution of caustic soda before reassembling.

It may be pointed out that the actual volume of oxygen absorbed by the blood passing through the machine can be determined by having a closed air circuit under constant pressure and temperature conditions and measuring the volume changes which occur. The CO_2 would of course have to be removed as liberated, the partial pressure of the CO_2 being determined by some instrument such as the katharometer (Daynes(8)). It is also necessary to keep the speed of rotation constant so that the volume of blood in the machine, and consequently the air volume, will not alter.

SUMMARY.

An artificial "lung" consisting of alternate revolving and stationary plates is described which, in an atmosphere of air, is capable of introducing into the blood flowing through it 0·8 c.c. of oxygen per minute per each pair of plates. The rate at which oxygen is introduced is not altered by variations in blood flow up to 260 c.c. per minute. Such an oxygenator consisting of five pairs of plates has an air volume of 600 c.c., and when revolving at a speed of 200 revolutions per minute contains 54 c.c. of blood at any moment. When running in an atmosphere of oxygen it will introduce 19·0 c.c. of oxygen per minute into the blood flowing through it.

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THE RÔLE OF THE THYROID AND PARATHYROID GLANDS IN VASO-MOTOR REFLEXES.

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Introduction.

Beyond a few generalised statements referring to increased excitability or depression of motor, sensory, and visceral nerves on extirpation of the thyroid and parathyroid glands there is no literature dealing with the part normally played by these glands in the vaso-motor reflexes. There is moreover considerable discrepancy between the statements of different observers. Carlson and Jacobson(1) describe signs of depression rather than excitability, and Smith(2) also refers to paralysis of the vaso-constrictor mechanism of the blood vessels, whilst Falta and Kahn(3), Frankl-Hochwart(4) and others have postulated hyper-excitability.

We suggest that these conflicting views are held because of lack of information of the normal responses from nerve terminals under varying conditions.

Having completed an investigation of the normal vaso-motor reflexes resulting from stimulation of the nerve terminals in different tissues (Vincent and Thompson(5)), we have turned our attention to an investigation of the parts played, if any, by the various endocrine glands in the body in relation to vaso-motor reflexes and associated phenomena, and the present paper deals with our experiments relating to the thyroid and parathyroid glands in particular.

Methods.

All the experiments have been performed upon decerebrate cats. The main venous return from the thyroid and parathyroid glands is *via* a large vein formed by the junction of two efferent vessels from each set of glands. The main vein lies in close proximity to the trachea, usually occupying a ventral position, but frequently to be found laterally or even posteriorly. It is therefore of the utmost importance to avoid ligature of the vein when inserting a tracheal cannula, and possibly some of the

inconstancy of work done on animals is due to the fact that ligature of this vein is a matter of chance. Having ligatured the carotid arteries and removed the brain to the level of the corpora quadrigemina, the cut surface of the brain stem was covered with temporal muscle kept in place by a warm swab. We have found that this method of packing the cranial cavity is superior to our former method of tight compression by means of swabs, because no undue pressure is exerted upon the respiratory centre and the animals breathe normally and quietly after the operation.

At least one hour was allowed to elapse after decerebration before any experiment was performed, in order to eliminate all traces of anæsthetic.

An arterial cannula was then put in the carotid artery and the blood-pressure recorded by means of the usual mercury manometer.

Statement of results.

The normal vaso-motor reflex of skin and muscle was recorded in each experiment by scratching and kneading respectively before removal of the glands. The thyroid and parathyroid glands on both sides were then removed and the scratching and kneading performed at short intervals. No change in the results was detected until one hour (on an average) after extirpation of the glands. At that time it became obvious that any previous depressor response was either considerably diminished or superseded by a pressor reaction, and if the normal reflex had been pressor, that augmentation of it was in process.

After experimenting upon a long series of animals it was apparent that this period of time (one hour) was a critical one because it was marked by the sudden death of a large number of animals. The mode of death is peculiar. The minute before death the animal will be breathing regularly, the force and rate of the heart beats good, the blood-pressure high, and the animal apparently in good condition. Without warning, and without any convulsions or other signs of abnormal disturbance, the breathing will cease, the heart rapidly fail, the blood-pressure fall, and within a minute the animal is dead. This has occurred so many times at this period of the experiment with identically similar series of events that we have come to consider this as a crisis due to extirpation of the glands. We have also found that the crisis may be precipitated by stimulation of the skin, muscle, or intestine.

A large number of animals, however, survive this critical period and show a remarkable alteration in the vaso-motor responses from the various tissues. If the normal reflex is depressor this eventually becomes a marked pressor effect, or if the normal response is pressor this is exaggerated.

The pressor response, when established, continues to be exaggerated to an astonishing degree, and a point of time is reached when the maximum occurs and is followed by a rapid decrease, thus exhibiting a second crisis, Fig. 1. It occurs usually about six hours after removal of the

Fig. 1. Scratching skin of abdomen at two hourly intervals after removal of the thyroid and parathyroid glands. The first tracing (reading left to right) indicates the effect of scratching the skin before extirpation of the glands. Note the progressive augmentation of the pressor response reaching a crisis six hours after the extirpation.

glands, but it appears to bear a relationship to the initial response. The greater the depressor response before removal of the glands, the sooner the crisis is reached.

Coincident with this second crisis the decerebrate cat shows several characteristic signs of tetany—rapid respiration, Fig. 2, hyper-excita-

Fig. 2. Tracings of the respiration of a decerebrate cat at hourly intervals after extirpation of the thyroid and parathyroid glands. *A* shows the normal respiration before removal of the glands.

bility of deep reflexes, and occasional muscular "twitchings." Blood removed from the animal at this stage clots very slowly.

This exaggerated pressor response is also very evident in stimulation of the intestine, Fig. 3. In cats surviving the first crisis kneading of the gut four hours after extirpation of the glands results in a large rise of blood-pressure. Although the intestine is exposed to the air and handled frequently the pressor response still prevails without any signs of the

normal fall of blood-pressure supervening, which, as we have shown, invariably occurs when the glands are intact and the gut is exposed and

Fig. 3. Successive kneadings of exposed gut producing marked rises of blood-pressure without any depressor effect occurring. Thyroid and parathyroid glands removed four hours previously.

frequently handled. Removal of the semi-lunar ganglia and section of both vagi does not affect the result.

An experiment was tried in which one gastrocnemius muscle was denervated before extirpation of the glands, and the other gastrocnemius muscle left intact. At this stage a fall of blood-pressure resulted from kneading the intact muscle whilst a slight rise occurred on similar stimu-

Fig. 4. Kneading of intact gastrocnemius muscle *A* and denervated gastrocnemius muscle *B* three hours after extirpation of the thyroid and parathyroid glands.

lation of the denervated muscle. Three hours after extirpation both gastrocnemii muscles elicited the same degree of pressor response showing that the augmentation of the pressor effect is not due to depression of the vaso-motor reflex, Fig. 4.

Moreover, stimulation of the skin, muscle, or intestine at short intervals of time results in an exhaustion of the pressor response which re-appeared after an interval of recovery, Fig. 5.

Fig. 5. Repeated stimulation of skin four hours after removal of the thyroid and parathyroid glands showing exhaustion, and re-appearance of the rise of blood-pressure after an interval for recovery.

Administration of calcium salts. Injection of various salts of calcium (chloride, lactate, etc.) in varying strengths at different points in the experiments has failed to alter the results in any way. The "reversal" process takes place in the same fashion, Fig. 6. We should like to

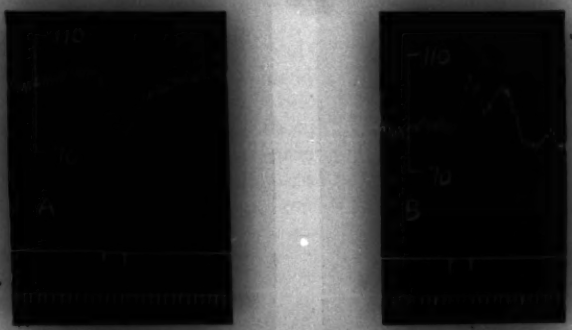

Fig. 6. *A*, stimulation of skin before removal of glands. *B*, stimulation of skin after removal of glands and administration of calcium salts.

interpolate here that we have observed the occurrence of a curious phenomenon in cats when injected with solutions of calcium chloride a few hours after the thyroid and parathyroid glands have been removed. The animal commences to perform automatic rhythmical movements

and continues to do so for a remarkably long time. For example, one cat executed all the movements manifest in the exercise of running and continued to do so for over two hours.

Administration of parathyroid extract (Collip). 0·2 c.c. of parathyroid extract (Collip) was injected at the time of extirpation of the glands, followed by second and third injections of the same quantity of extract at intervals of half an hour. A further injection of 0·2 c.c. was given two hours later. This treatment completely prevented the "reversal" reaction, and the animal four hours after the removal of the glands still showed the same fall of blood-pressure on stimulation of the skin or muscle which it exhibited before the extirpation. In the case of the intestine the usual depressor effect was evident.

But when the first injection of extract was not administered until the "reversal" or augmentation had become established it was much more difficult to abolish the pressor effect. Larger and more frequent doses were necessary, and in some instances no change could be effected until at least 1·5 c.c. of extract had been given.

Double adrenalectomy. Ligature of the adrenal veins produced no change in the course of events we have described as following removal of the thyroid and parathyroid glands, Fig. 7. It is becoming increasingly

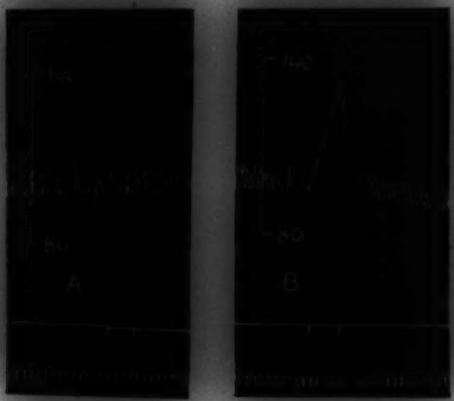

Fig. 7. *A*, effect of stimulating the skin before extirpation of the thyroid and parathyroid glands. *B*, effect of stimulating the skin three hours after extirpation of the glands and ligature of the adrenal veins.

clear to us that ligature of the adrenal veins is a most ineffectual method of eliminating adrenal secretions. There is a good collateral circulation

beneath the gland and it is necessary either to ligature the whole glands or remove them, if complete abolition of adrenal function be desired.

Therefore, both adrenal glands were removed at the same time as the thyroid and parathyroids. This also prevented the depressor reflexes being replaced by pressor responses or exaggeration of the initial pressor effects upon the blood-pressure, Fig. 8. If, however, the adrenal glands

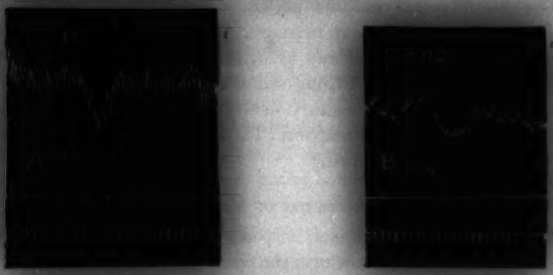

Fig. 8. *A*, stimulation of skin before removal of thyroid and parathyroid glands. *B*, stimulation of skin four hours after removal of thyroid, parathyroid and adrenal glands.

were removed later when the second crisis was approaching, the rises of blood-pressure still persisted although very considerably diminished in size.

It has been possible to keep several decerebrate cats alive twelve and fourteen hours, and to study the condition after the second crisis has passed. In a few cases the original vaso-motor reflex—the depressor response—returned, the depth of the fall in blood-pressure being much greater than before the glands were removed. Examination of the adrenal glands at this stage revealed them as dull grey in colour instead of the normal yellow tint. Staining with potassium bichromate showed that they were exhausted of adrenaline, and a cold saline decoction of the glands injected into an animal gave only a very slight rise of blood-pressure.

Cats in a state of experimental tetany. Thyro-parathyroidectomy was performed, and animals developed complete tetany on the third or fourth days after operation.

All the animals examined in this series reacted in the same way provided they were decerebrated before the onset of violent tetanic spasms. If decerebrated when in the stage of "fits" the animal rapidly succumbed. The time chosen for the experiment was therefore the period immediately preceding the convulsive stage.

The animal was decerebrated in the usual way. During the hour allowed for elimination of anæsthetic the animal behaved normally. As soon as stimulation of tissues began violent fluctuations of blood-pressure resulted, the pressor effects markedly predominating. Very irregular tracings were therefore obtained. This continued for about half an hour and came to a sudden termination. Within one or two minutes the blood-pressure tracing became perfectly regular and was sometimes coincident with a fall of mean pressure. Stimulation at this stage failed to provoke any response of any kind, no matter how intensive or extensive the stimuli were. Strong stimulation of the central and peripheral ends of motor and sensory nerves (sciatic, anterior crural, etc.) failed to effect the slightest response. The cat continued in this condition for a short time and then died.

Discussion of results.

That the exaggerated pressor response is due to an increased liberation of an autacoid substance into the circulation is rendered evident by the fact that it can be exhausted by repeated stimulation, re-appearing after an interval for recovery, and by its general behaviour in the series of experiments performed. We have already given full proofs that the pressor response elicited when tissues are stimulated is due to liberation of an autacoid pressor substance, and it is this part of the response to stimulation which is exaggerated when the thyroid and parathyroid glands are extirpated.

A possible explanation of the augmented pressor response is that the vaso-motor reflex is in some way inhibited and thus the autacoid substance has unlimited action. Against such a theory is the fact that the increase is progressive and attains very large proportions. But our work with the denervated and intact muscles shows clearly that there is no depression of the vaso-motor reflex, otherwise the response in the intact muscle would have been much the greater.

Moreover, in animals surviving for some hours beyond the second crisis the vaso-motor reflex is again in evidence upon stimulation.

Therefore we consider that when the tissues are stimulated in the absence of the thyroid and parathyroid glands there is an increased amount of the autacoid substance which raises the blood-pressure.

The fact that Collip's extract under certain conditions completely abolishes this variation of the normal reactions suggests that the parathyroids are largely concerned in the matter. Supporting this contention is the coincidence of characteristic signs and symptoms of tetany at the time of the second crisis when the pressor reaction is most marked.

Also, it is very significant that cats in a condition of experimental tetany exhibit only marked pressor responses.

This, then, leads us to believe that the parathyroid glands in particular exert directly or indirectly some inhibitory control of the autacoid substance liberated in the tissues upon stimulation. This control may be metabolic, directly antagonistic, or indirectly antagonistic by neutralisation of some other substance normally stimulating secretion of the autacoid pressor substance.

In the solution of this problem it is important to note that parathyroid extract, while being a perfect preventive reagent of the variation from the normal when administered in sufficiently large doses, is only a poor curative reagent once the abnormal condition is established. If the parathyroid glands by virtue of their extract control the metabolism of this autacoid substance found in tissues, one would expect the extract of these glands to find no such great difficulty in curing the condition, as is true in fact. It is interesting, too, that calcium salts utterly fail to affect the series of phenomena following extirpation of the glands which are supposed to control calcium metabolism.

Again, if the rôle of the glands was directly to antagonise formation of the autacoid substance, a much quicker action would be expected.

On the other hand, the absolute prevention of the presence of abnormal quantities of autacoid pressor substance in the circulation by parathyroid extract administration, and the prolonged difficulty of controlling the excess when present in parathyroidectomised animals, is strongly suggestive of the presence of a substance in the blood stream which acts as a stimulant to the excretory organs of the autacoid pressor substance. When this "stimulant" has unhindered action, administration of extract has greater work to do in inhibiting its action due to accumulation, and the effects are delayed in manifestation owing to the large amounts of pressor substance already secreted in the tissues.

Such a conclusion is further corroborated, as we shall show later, both by our experiments which included double adrenalectomy and by those performed on cats in the stage of experimental tetany.

As already explained, ligature of the adrenal veins has, on account of collateral circulation, no effect, but double adrenalectomy produces definite and distinctive results. That double adrenalectomy performed at the same time as the thyroparathyroidectomy prevents any increase in autacoid pressor substance, indicates an active participation of these glands in the production of this autacoid substance. Several other results in our experiments contribute to this indication. For instance, the obvious exhaustion of both adrenal glands immediately after the

second crisis, and the rapid and marked diminution in the rises of blood-pressure consequent upon stimulation after removal of both adrenal glands. Small rises still result due to accumulation of autacoid pressor substance.

We have therefore come to the conclusion that the exaggerated pressor response is due to the formation and liberation of large quantities of an autacoid pressor substance probably due to stimulation by some substance secreted by the adrenal glands, which is normally inhibited in its action by the parathyroid glands, and possibly by the thyroid gland.

The rôle of the adrenal glands is a matter we are at present investigating.

From the results of the present investigation we feel justified in concluding that one of the functions of the parathyroid glands (and possibly to a small extent the thyroid gland) is to limit the secretion of autacoid pressor substance in the manner already indicated.

Summary.

Decerebrate cats in which the thyroid and parathyroid glands are removed show after a certain lapse of time an abnormally large pressor response upon stimulation of skin, muscle, or intestine. Two crises are described as occurring in the process.

Under certain conditions this abnormality can be combated successfully by the injection of parathyroid extract (Collip), and also by double adrenalectomy.

Reasons are advanced to show that the increased pressor response is due to liberation of large quantities of autacoid substance and is not owing to any depression of the vaso-motor reflex. Further, we consider the secretion of this autacoid substance to be stimulated by some substance liberated by the adrenal glands, and that one of the functions of the parathyroid glands (and possibly the thyroid also) is to limit the secretion of autacoid pressor substance by control of the aforesaid stimulating agent.

We beg to acknowledge our indebtedness to the Ductless Gland Committee of the British Association for a grant to one of us (S. V.).

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

July 14, 1928.

The effects of breathing CO_2 and O_2 mixtures upon the CO_2- and O_2-tensions in the tissues. By J. ARGYLL CAMPBELL.

Normal unanæsthetised rabbits (2–3 kilos) were fixed comfortably on their backs and rebreathed the mixtures of CO_2 and O_2 from a Douglas bag containing about 100 litres. The snout of the rabbit was within the bag, leakage being prevented by means of a rubber ring fixed securely into an opening in the bag and fitting closely to the vaselined snout of the animal. The main object of the experiments was to determine whether in breathing O_2 containing 5 p.c. CO_2 (as advocated by Yandell Henderson) the presence of CO_2 would in any way interfere with the entry of O_2 into the tissues. The tissue CO_2- and O_2-tensions were estimated by the method of injection of 200–300 c.c. of N_2 into the abdominal cavity and under the skin. Four rabbits were employed and three series of two observations were made with each, *i.e.* 24 in all, care being taken to have approximately the same amount of gas present in the tissues of the same animal for each series of experiments. In Series I the animals rebreathed O_2 (60–90 p.c.) only, the CO_2 produced by the animal being removed by circulation of the gas through a soda-lime container. In Series II, O_2 was rebreathed without the CO_2 being removed so that in 60 minutes the CO_2 pressure in the inspired air had risen to 14·5 mm. Hg and in 120 minutes to 31·0 mm. Hg. In Series III the animals rebreathed O_2 plus about 5 p.c. CO_2 (35·5 mm. Hg.) so that in 60 minutes the CO_2 pressure in the inspired air had risen to 43·2 mm. Hg and in 120 minutes to 56·7 mm. Hg. In the Table the letter *A* refers to the first 60 minutes of experiment and the letter *B* to the first 120 minutes of the experiment. The normal CO_2-tension in the tissues of the animals was about 45–50 mm. Hg and the normal O_2-tension about 40 mm. Hg in the abdominal cavity and 20–25 mm. Hg. under the skin. The results in the Table give the average differences, produced by the experiments, in mm. Hg and also in percentages of normal.

It will be observed that the presence of CO_2 in no way prevented the entry of O_2 into the tissues, the expected rise of O_2-tension in the ab-

dominal cavity being as marked when breathing the mixture with 5 p.c. CO_2 as when CO_2 was absent. In all cases there was no change produced in the O_2-tensions under the skin in the time allowed for the experiment, but in previous observations (1) of much longer duration a definite rise was observed frequently in this region when breathing O_2 at high pressure.

The presence of CO_2 in the mixture breathed of course markedly affected the CO_2-tension in the tissues, the average increase in 2 hours being as much as 42 p.c. in the abdominal cavity (see Series III *B*), that under the skin being 23·8 p.c.; the smaller increase under the skin is simply due to the fact that, with this type of experiment, the changes under the skin lag behind those in the abdominal cavity, probably because of poorer circulation under the skin.

Average differences in tissue tensions of four rabbits.

Expt. Series No.	Press. in air breathed, mm. Hg.				Tissue tensions, Increase (+) or Decrease (−) compared with normal. CO_2-tension				O_2-tension			
	CO_2		O_2		Abd. cav.		Skin		Abd. cav.		Skin	
	start	finish	start	finish	mm. Hg.	%	mm. Hg.	%	mm. Hg.	%	mm. Hg.	%
I *A*	0	0	463	454	+ 0·2	+ 0·5	+ 0·2	+ 0·5	+2·2	+ 5·9	+0·0	+0·0
II *A*	0	14·5	481	457	+ 4·0	+ 8·9	+ 3·2	+ 6·8	+6·0	+14·4	+0·5	+3·9
III *A*	35·5	43·2	451	442	+ 8·0	+17·4	+ 4·7	+10·4	+5·0	+12·4	−0·7	−2·9
I *B*	0	0	463	443	+ 1·2	+ 2·7	+ 1·5	+ 3·1	+5·0	+13·9	+0·5	+1·2
II *B*	0	31·0	481	439	+ 9·0	+20·0	+ 5·7	+12·1	+3·0	+ 7·8	−0·7	−2·8
III *B*	35·5	56·7	451	416	+19·2	+42·5	+11·0	+23·8	+5·2	+14·1	−0·5	−1·6

Note on sweating. By W. Burridge.

In the warm weather shade temperatures on the Indian plains vary from 40° C. to 52° C. A journey taken while such high temperatures range, by motor for example, is at first pleasant, but after some time the traveller begins to appreciate he is becoming dry. If a drink be taken after a sufficient degree of dryness has developed, sweat breaks out before even a glass of water can be hurriedly drunk.

The sweating begins on the forehead and spreads thence over the head, neck, arms and trunk. It is associated with a peculiar sensation, possibly similar to that popularly associated with states of fear and known as the "creeps."

The milder degrees of dryness give sweating after a lapse of some 30 seconds, provided water has been drunk, without any appreciable sensation. One can find indeed all types of variation between sweat accompanied by "creeps" breaking out while water is being drunk, and

(1) Journ. Physiol. 63. p. 325. 1927, etc.

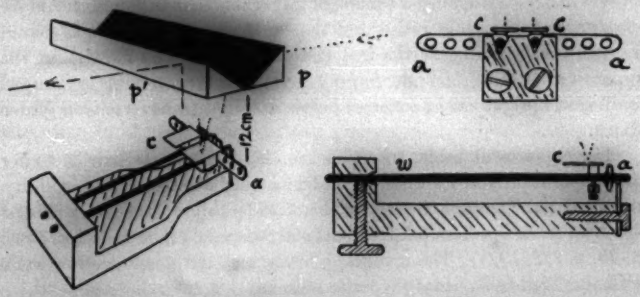
sweat coming out without sensation some time after water has been taken.

The sweating outburst with "creeps" is evidently a sympathetic action previously inhibited by lack of water. The short reflex time possible —5 seconds—is also too slow for the water drunk to pass to the sweat glands. It would seem, then, that water in the alimentary canal is a factor in the body's water balance.

Sweating may also be conditioned by the surroundings, *e.g.* one may be in relative comfort in a large room in hot weather, and yet sweat profusely immediately after entering a smaller adjoining and communicating room. These differences in body reaction to the large and small room are well known in the tropics and attributed to the differences in radiant heat received by the body from the walls and roof. It would seem impossible, however, for the body temperature to be raised appreciably by the mere passage from one room to another, so that this sweating would appear to be a reflex with its receptors in the skin.

A mammalian myograph. By C. S. SHERRINGTON.

The arrangement is as follows: The magnified image, obtained by a low-power microscope objective, of an illuminated rectilinear slit, similar in pattern to a spectrometer slit, is thrown vertically across the horizontal slit-opening of a cardioelectrograph camera (Cambridge Inst. Co. pattern)



upon a falling photographic plate. On its way from the objective to the plate the image is deflected downwards some 12 cm. by a rectangular prism (*p* in figure) upon two little horizontal mirrors *c.c*, one attached to each of the two parallel and horizontal wires *w* forming the double torsion myograph. The mirrors return their images to a right-angled prism *p'*

adjoining the previous one, and thence the two images of the slit pass through a cylindrical lens which condenses them for the camera-plate. Torsion of either wire displaces its member of the image pair horizontally on the falling photographic plate. For torsing the wire the muscle, rigidly clamped to the experimental table below, is attached by a light steel hook to a short lever arm, *a*, brazed to the wire at right angles to the wire's axis. The steel torsion wire is supported and set as in the isometric "shadow"-myograph for mammalian work previously described (*Roy. Soc. Proc.* B. **92**, p. 245, 1921), but carries at some desired distance, *e.g.* 15 mm. from its clamped end, a minute first-surface (rustless steel) mirror braced to it by a sessile ring.

With the camera at convenient distance (*e.g.* 1 metre) from the preparation and with the muscle tendon attached at 4 mm. from torsion wire axis, the excursion of the image on the plate amplifies by somewhat more than 650 times the movement of the tendon. For a tension-myogram of even 50 mm. excursion the angle of torsion need not then be more than 1° and the shortening of the muscle (*e.g. semitendinosus* or *tibialis anticus*) will not be more than $\frac{1}{700}$ of their length. Isometrism as a condition is therefore approximately complete. The range of tension-scale employed can by using steel wire of different gauge, or of different length, easily be varied, *e.g.* from 100 grm. per 1 mm. excursion to 3 grm. per 1 mm. excursion. The natural vibration period of the system has been with the wires used more than 1500 per sec. Sudden complete release from a tension of 6·5 kilos against a torsion of rather less than 2° found the image steady again at zero in rather less than ·015 sec. Damping is favoured by the V-slot bearing at the free end of the wire. The fixation of the apparatus has to be rigid: the frame carrying the torsion-wires is clamped to an iron upright set in concrete below and in a transverse iron girder above.

The experiment table, to which are clamped the steel drills fixing the preparation and its limb, is, and must be, constructed for solidity and rigidity. The table in use weighs considerably over 1 cwt.; it is of the "fall-table" pattern (cf. Liddell and Sherrington, *Roy. Soc. Proc.* B. **96**, p. 212, 1924). The myograph lends itself extremely well to work with the "fall-table" and myotatic reflexes.

Three years experience with this myograph has proved its suitability for dealing reliably with the speedy and often very considerable tension-changes of warm-blooded muscle (cf. Creed and Sherrington, *Roy. Soc. Proc.* B. **100**, p. 258, 1926; Cooper, Denny-Brown and Sherrington, *ibid.* p. 448, 1926, and **101**, p. 262, 1927). Recording a bright line on a dark

field, it is, otherwise, a modification of the "shadow"-myograph earlier described (Sherrington, *Roy. Soc. Proc.* B. **92**, p. 245, 1921). It presents certain advantages: rectilinear instead of slightly curvilinear ordinates, smaller torque of the steel resistance, and owing to higher magnification the attainment of fuller isometrism. Also, owing to the darkness-background, the same plate can be used without practical detriment to clearness, for several, *e.g.* a dozen successive exposures. But this form of the myograph does not lend itself as does the "shadow"-myograph to combination with the string-galvanometer (Fulton, *Roy. Soc. Proc.* B. **96**, p. 475, 1924), a shadow instrument. It provides by its two wires for concurrent records from two muscles if desired, as is also done by the shadow myograph in its modification due to Denny-Brown (*Roy. Soc. Proc.* B. **102**, in the press). As light source for this system an incandescent filament lamp suffices, even for plate-travel of 40 cm. per sec.; an arc-source is not required.

The osmotic pressure of egg albumin. By John Marrack and Leslie Frank Hewitt. (Introduced by Prof. Roaf.)

We have measured the osmotic pressure of isoelectric egg albumin, using solutions of NaCl buffered with sodium acetate and acetic acid as our external fluid.

With concentrations up to 0·12 molal and at 5·9 molal the pressures found agreed closely with those found by Sörensen (1917) with $(NH_4)_2SO_4$ up to 0·33 molal as external fluid.

The fall of osmotic pressure with hydrogen-ion concentrations above that of the isoelectric point found by Sörensen in the presence of $(NH_4)_2SO_4$ did not occur in the presence of low concentrations of the buffer solutions.

The state of aggregation of egg albumin appears little changed by alteration in the nature of the salt present, and, within limits, of the concentration of the salt.

The molecular weight calculated from Sörensen's and from our figures is 43,000 (cf. Adair (1927)); this does not agree with Svedberg and Nichols (1926), and the discrepancy would not be explained by the amount of impurity they suggest.

In dilute solutions these results agree with the ideas developed by Scatchard (1927) on the neutral effects of salt and non-electrolyte on their activities.

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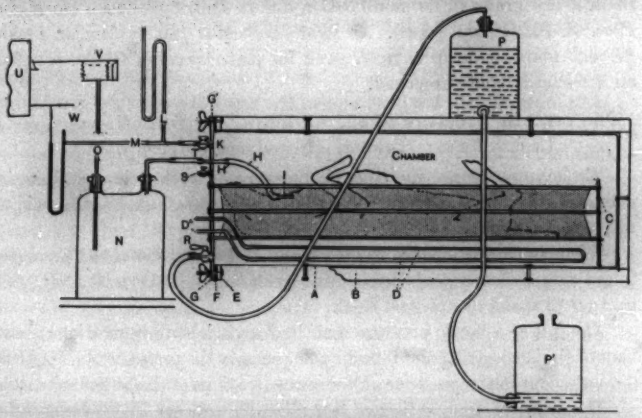
Method of investigating the effects of distension and collapse of the lungs on the respiratory rhythm in animals.

By Wm. H. Wilson and M. Hammouda.

The method was devised originally for the purpose of observing changes in the volume of the lungs brought about by positive or negative pressures acting on the surface of the body, in dead animals.

The apparatus was found to provide an accurate method of observing in living animals the reflex effects of such changes.

The apparatus consists of a metal chamber[1] (bolted to wood casing) with an air-tight cover *G*, *G'*, to which all the working parts, including the



carrier *C* for the animal, are attached. Through it pass the tracheal and other connections with the apparatus outside the chamber. The cover can be quickly detached and the animal thus removed from the chamber and reinstated without disturbance or interference with the continuity of the record. The carrier consists of 5 metal rods bolted to the cover at one end and a rest at the other; to the upper rod on each side is fixed a fold of cloth which serves as a sling in which the animal lies. The rate and volume of breathing are recorded by a simple bellows (*V*, cap. 700 c.c.) capable of recording a rhythm of 300 per minute.

The pressure changes in the chamber are effected by a head of water between the two bottles *P* and *P'*. The pressure is observed with a water

[1] Capacity after deducting contents including animal, about 30 L.

manometer *L*, recorded by a mercury manometer. The dead space between the trachea and air in the bottle *N* is about 20 c.c. which represents the dead-space of the upper air-passages. Two connections are not shown in the figure, one for a thermometer, the other for attaching the bellows if the box is used as a plethysmograph.

Method of operation. The animal is anæsthetised with chloralose or decerebrated. A tracheal cannula is introduced and the vagi freed. It is placed in the carrier, the tube *H* connected to the outlet *H'* and the carrier pushed into the chamber which is then closed. The release cock *S* being open and cock *R* closed a record of the breathing is taken.

With the bottles *P* and *P'* placed as in the figure, opening *R* (*S* being closed) produces a $-P$ in the chamber with inflation of the lungs, reversing the position of the bottles a $+P$ with deflation; the desired pressure being gauged by the water manometer when opening *R*.

The effects on the reactions of excess CO_2 can be examined by adding the required percentage to the air in bottle *N*, a deficiency of oxygen by placing a CO_2 absorber in the circuit.

Comparative observations of the reactions after vagotomy have been made. The procedure of withdrawing the animal, dividing the vagi and replacing it takes about three minutes, the continuity of the record being unbroken. To observe the respiration during recovery after an observation the bellows is attached direct to the chamber which functions as a plethysmograph. After each observation the air in bottle *N* is renewed, the animal breathing meanwhile to the open air.

The method appears to offer the following advantages:

(1) Distension and collapse of the lungs is brought about in a manner imitating the natural process.

(2) The rate of rhythm, duration and form of the phases of the respiratory cycle, ventilation and changes of tonus are accurately recorded and can be measured.

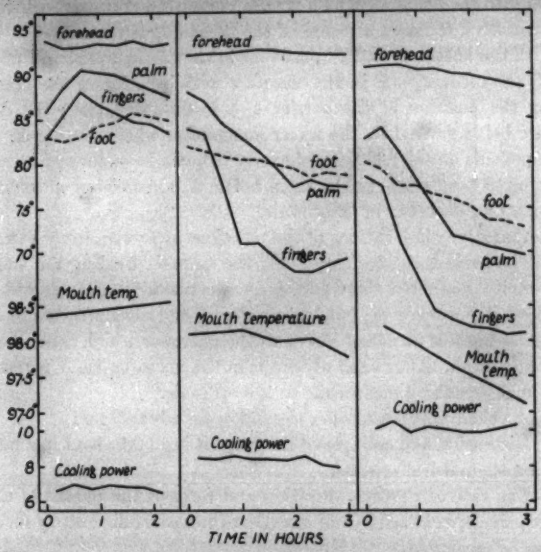
(3) The duration and extent of the response can be correlated with the strength of the stimulus (degree of inflation or deflation).

(4) By a comparison of the response to inflation under various conditions a measure of the effect of these conditions on the excitability of the centres is obtained.

It is to be noted that in the method all reflexes from the respiratory tract above the trachea are excluded.

The influence of various systems of artificial heating on skin temperature. By H. M. VERNON, T. BEDFORD and C. G. WARNER.

In most systems of artificial heating the floor and the air at foot level are cooler than the air at head level; but some buildings are now heated by means of hot water pipes running under the whole of the floor, and it has been maintained that the increased warmth to the feet thereby effected enables a considerably lower air temperature to be supported with comfort. In order to obtain evidence on the point, the investigators



sat for periods of three hours at a time in a floor-heated room 24 × 21½ ft. in area. The flooring of composition tiles was maintained at a temperature of 63° to 77° F. Parallel observations were made in a room heated by an anthracite stove, direct radiation from the stove to the subjects of experiment being prevented by the interposition of a screen. The skin temperature was taken by means of a Moll thermopile, the cone of which was held within ¼ in. of the skin surface under examination. The unipivot galvanometer connected with the thermopile attained its maximum deflection in 7 sec., and its readings, based on careful calibrations, enabled the skin temperature to be ascertained with accuracy. The temperature

was taken of the forehead, the upper surface of the foot near the web of the toes, and the palmar surfaces of the hand and of the fingers.

The average results obtained in the floor-heated room, when the air (which was tested at a height of 6 in. and 4 ft. 9 in. above floor level) had a mean cooling power of 6·8, 8·3 and 10·1, are shown in the figure. Four sets of observations were made on each investigator under each set of conditions, and it will be seen that at a cooling power of 6·8 the skin temperatures remained fairly steady throughout, that of the forehead being about 93° F., and those of the other surfaces, 84° to 88°. The atmospheric conditions were felt to be rather too warm for comfort, and the mouth temperature rose slightly. Air at a cooling power of 8·3 was felt to be rather too cold for comfort, and it will be seen from the figure that though the forehead temperature remained steady, that of the extremities fell considerably, whilst the mouth temperature fell 0·3° F. Air at a cooling power of 10·1 was felt to be much too cold for comfort, and it will be seen that during the course of the three hours the mouth temperature fell 1·1°. The forehead temperature fell 2°, whilst that of the foot, palm and fingers fell 8°, 13° and 18° respectively. Owing to the cooling of the hands, manual dexterity was reduced, as was indicated by the rate at which a test (the assembly of bicycle chain links) was performed. The effect of the cooling was to increase the time taken over the test by 10 per cent.

Parallel series of observations were made in the stove room at cooling powers of 6·8 and 8·3. The temperature of the floor itself in the stove room was 16° to 19° cooler than that of the floor-heated room, and it was found that at the end of the 3-hr. sessions the skin temperature was distinctly lower than in the floor-heated room. That of the face was 0·7° less, that of the foot 3° to 5° less, of the palm 1° less, and of the fingers 3·7° less. It was calculated that at equal cooling powers the air of the stove room felt 2·5° cooler than that of the floor-heated room, or supposing that the temperature at head level were alone considered, the air in the stove room felt 5° cooler than that of the other room; *i.e.*, floor-heating did have a substantial influence in creating a sensation of warmth.

Pituitrin and the chloride concentrating power of the kidneys.

By J. B. S. HALDANE.

As Starling and Verney(1) have shown that without pituitrin the kidney cannot concentrate chloride, it seemed possible that the kidney's normal concentrating power might be increased by the injection of pituitrin. I therefore drank 20 to 40 grm. NaCl in 8 p.c. or 10 p.c. aqueous solution, after subcutaneous injection of 2 c.c. of a pituitrin solution sufficient to delay for about 8 hours the diuresis due to drinking 2 litres of water. The results were compared with those of control experiments without pituitrin. There was no definite effect on the urinary volumes. The maximum chloride concentrations reached were also little affected. In three pairs of comparable experiments the maxima reached were ·330, ·333 and ·313 molar without pituitrin, and ·326, ·316 and ·300 after injection. These changes are probably not significant. They are far less than those found by Stehle and Bourne(2) in dogs which had only drunk 2 p.c. NaCl solution, and were therefore probably not concentrating chloride maximally.

Hence the kidney's maximum chloride concentrating power, which incidentally has not varied during six years, was not affected by pituitrin-injection. Either the effect is wholly on the threshold, or the amount normally present in the blood is sufficient to allow the kidneys to concentrate maximally. Pituitrin injection had no marked effect on the volumes excreted after ingesting 50 grm. dry urea, or 4 litres of 1 p.c. NaCl solution.

(1) Starling and Verney. Proc. Roy. Soc. B, 97. p. 321. 1925.
(2) Stehle and Bourne. This Journ. 60. p. 229. 1925.

PROCEEDINGS
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PHYSIOLOGICAL SOCIETY,
October 13, 1928.

The placental glycogen. By A. St G. Huggett.

The maternal portion of the rabbit's placenta contains glycogen. This is maximal in quantity at the end of the third week of pregnancy as was shown by Lochhead and Cramer. They showed there is a parallelism between the growth of the fœtus and the quantity of glycogen in the placenta. In this paper attention has been directed to factors which modify the quantity of glycogen in the placenta at the 21st day and to the relation between the maternal liver glycogen and the placental glycogen. It is shown that percentage of glycogen in the placenta is unchanged by starvation, by carbohydrate feeding, or injections of carbohydrate or hormone. Insulin in 2 unit per kilog. doses has no effect, but repeated injections of bigger doses on successive days slightly lower the percentage from 3–4 per cent. to 1·5–2 per cent. Adrenaline injections have no effect on the placental glycogen though markedly diminishing the maternal liver glycogen. Thyroid feeding for six days caused a 10 per cent. loss of weight, marked reduction of the liver glycogen but hardly any effect on the placental glycogen. Repeated injections of phlorrhizin were shown by Lochhead and Cramer not only to arrest the deposition of glycogen but also to arrest growth. The following facts were therefore obtained. There is no relation between the deposition of glycogen in the maternal liver and the maternal placenta. Factors which increase or diminish the former have either no effect or a negligible effect on the latter. The only influence having any marked action on the glycogen of the maternal placenta is a profound disturbance of metabolism such as is produced by repeated injections of phlorrhizin, or insulin during several days' starvation, or by pathological changes.

Weights of hearts of fœtal and newly born animals. By M. S. Pembrey.

Differences in the weights of the left and right ventricles appear to be related to the immaturity or maturity of the animals at birth. The capacity for muscular coordination and work is a biological measurement,

weight of body is not. There are large individual variations which should be considered as physiological and related to adaptation. The differences in the work of the two sides of the heart before and after birth may not be the sole factor.

Left ventricle cgrm.	Right ventricle cgrm.	Difference cgrm.	Ventricular septum cgrm.	Total auricular tissue cgrm.	Weight of body grm.	Remarks
Rabbit						
6	8	− 2	5	4	27	1 day ♂
7	10	− 3	6	6	33	„ ♀ Same litter
5	6	− 1	2	2	33	1 hour ♀
8	10	− 2	3	4	32	1st day. „
12	14	− 2	4	2	38	„ „
13	15	− 2	4	4	43	„ „
5	8	− 3	2	2	28	„ „
7	7	± 0	4	5	43	„ „
Cat						
29	32	− 3	8	8	121	1st day. Same litter
25	29	− 4	8	12	117	2 days. „
13	12	+ 1	3	4	63	1st day. „
11	15	− 4	3	4	67	„ „
23	27	− 4	12	10	85	„ „
12	18	− 6	5	6	54	„ „
14	21	− 7	7	8	65	„ „
24	25	− 1	8	10	74	„ „
17	22	− 5	5	8	68	„ „
22	25	− 3	5	8	77	„ „
Dog						
108	77	+ 31	30	31	356	1st day. Same litter
86	76	+ 10	24	28	317	„ „
73	100	− 27	27	35	265	„ „
89	85	+ 4	32	28	315	„ „
71	74	− 3	27	25	248	„ „
Pig						
189	197	− 8	81	91	592	1st day. Same litter
154	124	+ 30	39	32	829	„ „
214	226	− 12	97	118	887	„ „
366	201	+165	87	103	915	2–3 days. „
232	193	+ 39	76	77	912	1st day. „
143	142	+ 1	69	51	591	„ „
253	190	+ 63	94	84	907	1–2 days. „
178	126	+ 52	58	57	634	„ „
195	175	+ 20	69	59	709	2–3 days. „
Guinea-pig						
5	6	− 1	3	4	31	Still born. Same litter
8	8	± 0	4	5	36	„ „
18	14	+ 4	4	6	84	1st day. „
15	15	± 0	6	5	87	Still born. „
13	14	− 1	6	5	94	„ „
Man						
36 %	40 %	—	24 %	—	2834 grm.	Full term. Still born. In Percentage of Total Ventricular Tissue
38	45	—	17	—	2154	
36	41	—	23	—	—	
33	36	—	31	—	—	
32	41	—	27	—	3061	
27	41	—	32	—	3288	

Note. The blood was removed from the heart with water and the muscle was dried with filter paper.

Apparatus for demonstrating nerve and muscle action currents.

By E. D. Adrian and D. W. Bronk.

The action current discharges which occur during the normal activity of sensory and motor nerves and those in the muscles in voluntary contraction may be readily demonstrated to large classes by converting the potential changes into sound waves. A short length of nerve is isolated and placed on electrodes leading to a three valve amplifier and the output of this is connected to a second amplifier with two power valves operating a loud speaker. The method was shown to the Society at the meeting on May 19th, 1928.

A convenient sensory nerve preparation is a small piece of skin from the back of a frog with the attached cutaneous nerve. The skin is touched with a brush (in an insulated holder) and at each touch the discharge of nerve impulses produces a loud crackling sound in the loud speaker. With very light stimulation the individual impulses can be detected as a series of clicks. The absence of artefacts can be shown by touching the skin at a point outside the area supplied by the cutaneous nerve. Sensory impulses produced by stretching a frog's muscle can be demonstrated in the same way.

In a mammal (decerebrate cat or rabbit under chloroform) the various nerves of the neck can be used to demonstrate the motor impulses of the respiratory discharge in the phrenic and recurrent laryngeal and the sensory impulses in the vagus and cardiac depressor. Impulses from cutaneous receptors can be shown in the saphenous nerve by blowing lightly on the hairs of the leg.

The muscle action currents in the forearm during voluntary contraction can be converted into sound in the same way, but we have sometimes found that the circuit through the subject and the amplifier acts as a rectifier of high frequency radio waves, with the result that the loud speaker may demonstrate the programme from the nearest broadcasting station instead of the electromyogram. This can be minimised by inserting a high frequency choke in the input circuit.

The three valve amplifier has been specially built to secure portability and insensitiveness to mechanical vibration. The valves are of the Cosmos AC/G type which have a rigid cathode indirectly heated. They are resistance-capacity coupled (anode resistances 100,000 ohms, condensers 1 mf.[1] and grid leaks 1 megohm—all standard components)

[1] Large coupling condensers are used to enable the amplifier to deal with slow potential changes without distortion. Condensers of ·05 mf. would be large enough if the amplifier were to be used exclusively for sound recording.

and run from a common high tension battery (dry cells). Although the voltage amplification is about 15,000 (with 200 volts H.T.) there is no instability if the input and output leads are kept away from one another. The valves are shielded from sound waves by enclosing them in a felt-lined wooden box which is placed in a felt-lined tin case (earthed) covered with three-ply wood. The batteries are outside. The second amplifier is of conventional type with transformer coupling and volume control.

The volume of sound obtainable is limited by the fact that if the full power of both amplifiers is used, the sound waves from the loud speaker may produce a synchronous vibration in the case of the first amplifier, leading to a continuous howl; but the sound produced by a single nerve impulse may be made quite audible to a large class without pushing the amplification to the point where howling occurs.

The use of a loud speaker has proved of great value as a supplement to optical recording in research work on sensory and motor impulses. It is often possible to detect a nerve discharge by ear when an optical record would be confused by slower oscillations such as those due to the electrocardiogram.

The ultra-violet fluorescence of urine. By B. T. SQUIRES.

The fact that normal urine exhibits a blue-green fluorescence on exposure to ultra-violet radiation was noted by Jaffe[1] half a century ago. Langecker[2] considered that familiar urinary constituents such as urochrome, uroerythrin, urobilin and the porphyrins were not responsible for it. Kinnersley, Peters and Squires[3] found that the urochrome fraction prepared from urine by Garrod's technique fluoresces blue when diluted to a pale yellow. The literature, otherwise, is scanty.

Attempts have been made to investigate the relation between the output of fluorescent substances and that of other urinary constituents in human urine.

Method. The fluorescence of the urine under investigation was compared with that of a stable fluorescent standard by means of a simple form of ultra-violet fluorphotometer[4]; two similar thin glass or quartz cells, containing the urine to be investigated and the standard, are exposed to ultra-violet light, and the emitted beams of fluorescent

[1] Jaffe. Virch. Arch. 47. 400. 1869.
[2] Langecker. Z. physiol. Chem. 115. 1. 1922.
[3] Kinnersley, Peters and Squires. Biol. Journ. xix. 3. 1925.
[4] Squires and Jeffree. Journ. Scientific Inst. v. 9. 1928.

light are passed through an optical wedge to a simple form of comparator. Within certain limits of dilution, the intensity of fluorescence is proportional to the concentration of the fluorescent substance.

Results. (1) The daily output of fluorescent material is quite independent of the volume of urine excreted, and bears no close relation to that of any of the familiar urinary constituents so far examined. These results are based upon a series of extended observations on eight individuals.

(2) The graph of daily output exhibits well-defined maxima and minima, the interval between consecutive maxima or minima being 3–5 days; in urine from a subject on a fixed diet and in nitrogenous equilibrium the maxima and minima still occur, but tend to be constant in magnitude.

(3) Large (2–3 times) temporary increases in the fluorescent output can be brought about by the ingestion of (*a*) large quantities of protein, (*b*) large quantities of greenstuff (1 lb.).

The ingestion of carbohydrate or fat has no apparent effect.

(4) In fevers, such as pneumonia, the fluorescent output rises to 3–4 times normal, then slowly subsides, but does not approach normal level until the physical signs in the lungs have cleared up. The characteristic maxima and minima are still shown.

Class experiments on enzymic synthesis. By H. D. Kay.

In class experiments with enzymes the emphasis is laid, almost inevitably, on their hydrolytic activities. This is true even when intracellular enzymes (such as certain tissue esterases, glucosidases and ereptases) are being demonstrated, which enzymes *in vivo* probably preside over equilibria which are far nearer complete synthesis than complete hydrolysis.

The experiments detailed here are modified from a recent paper[1] in order to show in a simple manner to students (*a*) the fact of enzymic synthesis, and (*b*) that the same equilibrium is reached from both the hydrolytic and synthetic side in a balanced reaction catalysed by enzymes. For advanced classes the experiments are capable of extension, *e.g.* investigation of the kinetics of the reaction, or the actual isolation of the synthesised product and direct demonstration of its hydrolysability in dilute solution by the same enzyme that synthesised it (for details of these latter experiments see original paper).

[1] Kay. Biochem. J. 22. p. 855. 1928.

The reaction catalysed is

$$Na_2HPO_4 + C_3H_5(OH)_3 \overset{\text{phosphatase}}{\rightleftharpoons} C_3H_5(OH)_2PO_4Na_2 + H_2O.$$

Required. 1. Extract of cat's intestinal mucosa. The excised small intestine (preferably the duodenum and upper half of the jejunum) is washed thoroughly with water, and the mucosa carefully scraped off in the usual way. One part of the scrapings is ground up with nine parts of water and a little sand, three drops of chloroform being added for each 10 c.c. The mixture is shaken and kept overnight at room temperature, ground up again without further dilution next morning and filtered slowly through cotton wool[1].

2. Pure glycerol; an aqueous solution of Na_2HPO_4 containing 3 mg. P per c.c.; aqueous solution of sodium glycerophosphate containing 3 mg. P per c.c., and at the same p_H as the sodium phosphate; 25 per cent. solution of trichloroacetic acid; Briggs' reagents for inorganic phosphate determinations.

3. Test tubes with well-fitting glass or rubber stoppers; filter funnels; no. 30 Whatman filter papers, 7 cm.; series of pipettes; 25 c.c. graduated flasks.

4. Constant temperature bath at 38°; colorimeter and suitable lamp.

Enzymic synthesis. In two test tubes, *A* and *B* (synthesis tubes), place 1 c.c. phosphate solution, 2·5 c.c. extract and 5 c.c. glycerol (at 38°). In two test tubes, *C* and *D* (controls), place 1 c.c. phosphate solution, 2·5 c.c. boiled extract and 5 c.c. warm glycerol. Shake all the tubes. Take at once from each tube 1 c.c., run into 5 c.c. water + 2 c.c. trichloroacetic acid, allow to stand for at least 10 minutes, filter into test tubes, and stopper. Place tubes *A*, *B*, *C* and *D* in bath at 38°. The glycerol prevents bacterial action.

At end of 24 hours or longer, take a second c.c. from each of *A*, *B*, *C* and *D*, and deal with it as with the first sample. Now put 5 c.c. from each of the eight filtrates into eight 25 c.c. flasks, suitably marked (preferably with *yellow* grease pencil). Using Briggs' method determine the amount of inorganic phosphorus colorimetrically against a standard containing 0·2 mg. P. A considerable decrease in the depth of colour will be observed in the filtrates from *A* and *B*, but little if any change in those from *C* and *D*. The colour difference between $A_{0\,\text{hrs}}$ and $A_{24\,\text{hrs}}$ is usually quite easily visible to the unaided eye, and may be measured in any colorimeter.

If 1 c.c. of the reaction mixture from each of *A* and *B* is now put into 5 c.c. of distilled water, and incubated in the presence of a little chloroform or toluene for a further 24 hours, the synthesised glycerophosphate

[1] In presence of chloroform the extract is stable for some weeks in the ice box.

is almost completely hydrolysed again. This may be demonstrated by adding 2 c.c. trichloroacetic acid to each tube, filtering, and comparing 5 c.c. of filtrate as before against the 0·2 mg. standard.

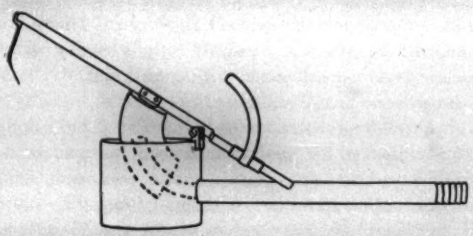
Equilibrium. To demonstrate the attainment of the same equilibrium from both the synthetic and the hydrolytic side, the enzyme solution should first be dialysed for 24 hours to remove diffusible phosphorus compounds. A series of four tubes is now made up. In *A* and *B* place 1·5 c.c. sodium glycerophosphate solution, 2 c.c. enzyme and 10 c.c. glycerol, in *C* and *D* place 1·5 c.c. sodium phosphate solution, 2 c.c. enzyme and 10 c.c. glycerol. 1 c.c. samples are taken, as before, immediately, and after 6, 12, 24, 48 and 72 hours (or other convenient intervals) at 38°. It will be found that approximately the same position of equilibrium (*i.e.* percentage of total P present as inorganic P, both glycerol and water being present in excess) is eventually reached in all four tubes.

A further similar, but rather more ambitious experiment, using 20, 40, 60 and 80 per cent. of glycerol in the reaction mixture, may be made by senior students to show that the final equilibrium position in a balanced reaction catalysed by enzymes depends on the relative concentration of the reactants.

By the above experiments it may be clearly and simply demonstrated that an enzyme will catalyse synthesis as well as hydrolysis, under conditions of concentration of the reactants which may possibly occur at active interfaces within the tissues.

A new volume recorder. By R. J. S. McDowall.

The principle is an old one, being that of the spirometer and other similar apparatus with a fluid seal, but by improvement of the design



friction is reduced to a minimum, while the curvature of the bell permits a large excursion of the lever. The centre of the curvature of the bell

and of the weight is at the fulcrum, and if the centre of gravity of the lever system is also exactly at the fulcrum the lever and bell are balanced at all positions, and for all practical purposes the entrance of equal amounts of air into the bell is recorded by equal excursions. The apparatus cannot leak. It is made by C. F. Palmer.

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November 10, 1928.

The resonance theory of audition: a historical note.

By J. H. Shaxby.

In the *Elementa Physicae* of Petrus van Musschenbroek (Leyden, 1729) appear the following paragraphs:

"§ 1176. ...Cochleae cavitus dividitur in duo cava, a se distincta ope sepimenti tenuissimi *XZ* (Tab. XXIII, Fig. 14), partim ossei *a*, *a*, *a*, partim membranacei *bbb*: hujus membranae fibrae decurrunt ab axe Cochleae ad exteriora, veluti Radii circuli a centro ad peripheriam, sunt proinde, a basi latiori ad apicem supputando, diversissimae longitudinis: quamobrem haec membrana est quasi Scala Triangularis, composita ex plurimis chordis tensis, diversissimae longitudinis."

"§ 1178. Quamobrem Auditus sequente modo peragitur:—(here follows a description of the passage of disturbance to the inner ear)—sed ea ("the mind") distinctissime cupiente cognoscere Tonos, tenditur membrana Tympani *G* (Tab. XXIII, Fig. 12) ope Musculorum Mallei, ut harmonice contremiscat, tenditur etiam ope Musculi Stapedis membrana fenestrae ovalis, ut et haec harmonice cum ossiculis tremat, et vehementius, quam a solo Aëre Tympani affici potuisset: tremente haec membrana, etiam Aër interior in Labyrintho *r*, tremet, qui afficiet nervos, expansos supra canalium semicircularium *O*, *P*, *Q*, internam superficiem, atque simul afficiet eos in Scala Cochleae *S*: Sonus vero quilibet in hoc Aëre excitatus, afficiet aliquot fibrillas *b*, *b*, *b*, Fig. 14, harmonice tensas, per §§ 1168 et 1169 hae Nervos, qui, hos motus per cerebrum ad Mentem deferentes, efficiunt, ut haec distinctissime Tonos Sonoros percipiat."

Paragraph 1168, here referred to, contains the following:

"Sint duae chordae musicae in eodem, vel in duobus Instrumentis, sibi proximis, unisonae; percussa una, sonabit quoque altera chorda, ejusque tremor ex imposito levissimo corpore ad oculum apparebit. Cum enim a tremente chorda Aër in undas cietur, hae incurrunt in alteram chordam, quam, quia haec pari velocitate contremiscere potest, facile in similes tremores commovent."

This paragraph shows clearly that what van Musschenbroek had in mind was not merely some vague "influence," but true resonance.

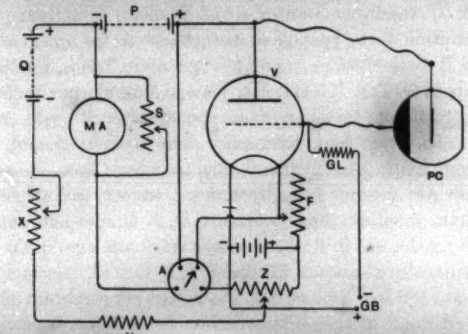
It is of interest to find at so early a date, not only mention of the increased tension of the ear-drum in attentive listening, but also a definite statement of the Resonance Theory of perception of notes of differing pitch, put forward independently and with precise detail by Helmholtz nearly a century and a half later.

The cadmium photoelectric cell as a means of measuring the absorption of ultra-violet radiation in dilute physiological fluids. (*Preliminary note.*) By F. Campbell Smith and C. Gordon-Wilson.

In a paper which appeared in the *Journal of Scientific Instruments* (1), D. T. Harris described a suitable circuit for using a thermionic valve to measure the change in potential produced at the cathode of a cadmium photoelectric cell when exposed to ultra-violet radiation.

It appeared to the present writers that such a combination could be used for the measurement of the absorption of ultra-violet radiation by dilute physiological fluids.

The circuit employed is shown in the figure. The valve used was an



V, Valve, P625 Osram.
PC, Cadmium photoelectric cell.
MA, Microammeter 0–240.
A, Three range meter:
0–150 volts.
0–7·5 volts.
0–30 milliamps.
GL, 5 Ω grid leak.
GB, Grid bias, 18 volts negative.
P, Anode battery, 140 volts.
Q, Compensating battery, 160 volts.
X, 20,000ω wire wound variable.
Y, 5,000ω wire wound variable.
Z, 400ω potentiometer.
S, 30ω shunt.
F, 20ω Filament Rheostat.

Osram P. 625 which has a high mutual conductance, thus giving the maximum change in plate current with the minimum change in grid potential. We consider this more sensitive and better adapted to the purpose, than the valve, a Marconi D.E. 5 b, used by Harris.

The source of radiation was a quartz mercury vapour lamp. It was found that its emission was far more constant if cooled by a suitable electric fan. The absorption cells were one centimetre in length.

To make a measurement two cells were used, one containing freshly distilled water, and the other the fluid to be tested. The microammeter reading for distilled water was taken as 100 per cent. transmission. A second reading was then made with the fluid under test, and the percentage transmission calculated.

If Beer's Law is obeyed, for the fluid under test, the concentration is proportional to the log of the transmission, therefore a standard curve can be constructed for a given substance, *e.g.* protein, which will correlate concentration with transmission. So far the radiation has not been monochromatic, and this introduces a source of error; but it is hoped to overcome this defect by the use of suitable filters (2). Unfortunately the cadmium cell is not sufficiently sensitive to allow of prismatic dispersion. Further work on these lines is being done and an attempt is being made to extend the method to the visible region of spectrum by the use of suitable photoelectric cells.

1. Harris, D. T. Journ. of Sci. Instruments, v. 5. 1928.
2. Svedberg. Journ. of Am. Chem. Soc. 48. 11. 1926.